Health Effects of Central Valley Particulate Matter

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University of California, Davis

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Work sponsored by

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Outline

- Study Motivation and Objectives (Dr. Pinkerton)
- PM Sample Collection, Extraction and Chemical Analysis (Dr. Bein)
  - Methodology
    - PM sampling; Filter extraction techniques; Chemical characterization
  - Comparative compositional analyses
    - Mass fractions; Mass closure; Retrospective mass reconciliation; Enrichment factors
- Animal Exposure Studies (Drs. Pinkerton and Tablin)
  - Animal exposure methods
  - Toxicological assay methods
  - Dose-response (PM extraction inter-comparison)
    - BALF, histologic evaluation, gene expression, immunohistochemistry, platelets
  - Time-delay (Urban versus Rural)
    - BALF, histologic evaluation, gene expression, multiplex cytokine assays, platelets
- Conclusions (Drs. Bein and Pinkerton)
  - PM extraction and chemical analysis
  - Dose-response
  - Time-delay

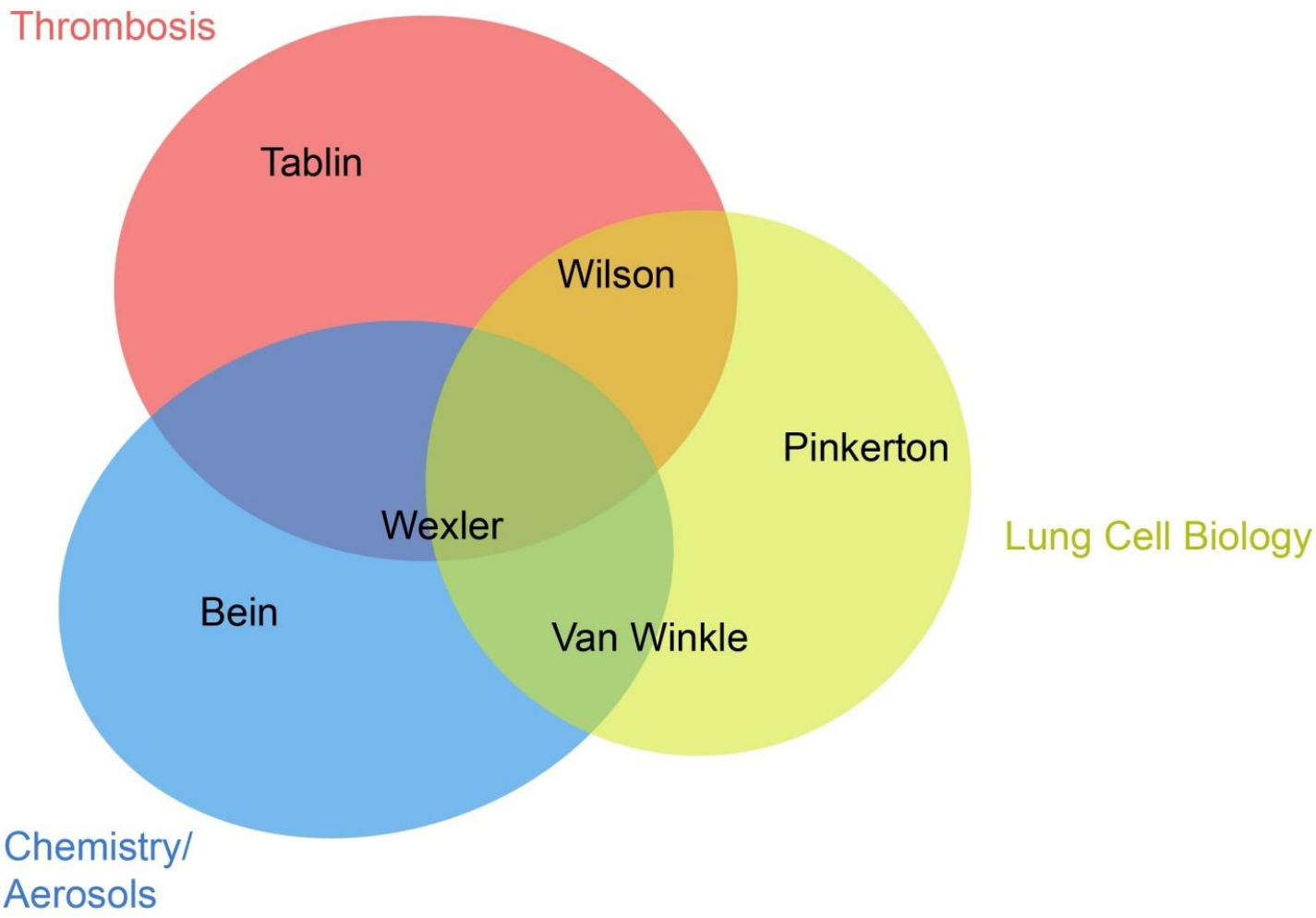
Study Motivation

- SJV Aerosol Health Effects Research Center
  - Five year EPA Health Effects Center at U.C. Davis
  - Found health effects associated with SJV Particulate Matter
- Questions Arising from EPA Health Center Studies
  - Are responses due to urban or background (rural) PM?
  - What is the dose-response relationship?
  - What are the time courses of the responses?
  - How do these time courses differ for
    - Pulmonary responses?
    - Systemic responses?

Study Objectives

- Comparative PM Toxicology and Mechanisms of Action
  - Urban versus Rural PM
    - Downtown Sacramento T&13th St. (Urban)
    - UC Davis Center for Health & the Environment (Rural)
  - Toxicological Endpoints
    - Pulmonary Responses
    - Systemic Responses
  - Dose Response
    - Intra-tracheal instillations
    - 10-100 μg PM in 50 μL delivery vehicle
  - Response Time Course
    - 1, 2, and 4 days post-instillation
- Bonus Objective
  - Comparative Assessment of PM Filter Extraction Techniques
    - Compositional and Toxicological
    - Multi-Solvent Extraction (MSE) versus Spin-Down Extraction (SDE)

Study Team


Thrombosis
Tablin
Wilson
Pinkerton
Wexler
Lung Cell Biology
Bein
Van Winkle
Chemistry/
Aerosols

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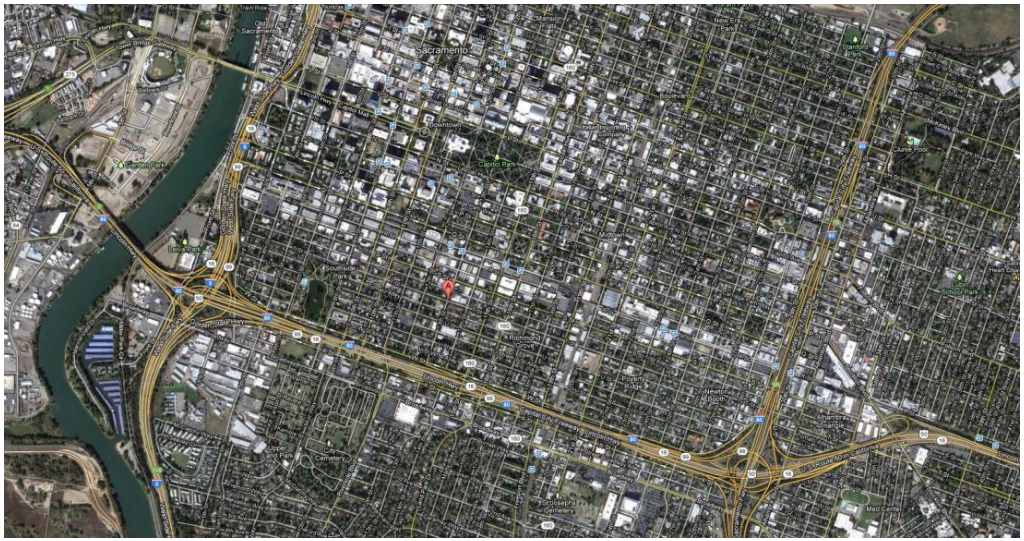
PM Sample Collection
Sampling Site



- Urban
  - Downtown Sacramento
  - NE corner of T St &13th St
  - On top of 2 story building
  - Source mixture
    - Vehicular (high traffic)
    - Residential (densely populated)
    - Commercial
    - Industrial

- Rural
  - UC Davis south campus
  - SE corner of CHE complex
  - On top of single story lab
  - Source mixture
    - Agricultural
    - Vehicular (low traffic)
    - Residential (sparsely populated)

PM Sample Collection
Sampling Equipment and Protocols

- Sampling Equipment
  - $PM_{2.5}$ High-Volume Sampler System
    - Equipped with PM_{10} size-selective head
    - Operated at 40 cfm
    - Coarse fraction ($PM_{10-2.5}$) collected on Al foil substrates
      - Pre-baked at 500° C for 24 hrs
    - Fine fraction ($PM_{2.5}$) collected on Teflon coated glass microfiber filters
      - Pre-cleaned via successive sonication in milli-Q H_2O, DCM and Hx
- Sampling Protocols
  - Field studies conducted simultaneously at both sites
  - Field studies conducted during winter and summer 2011
  - PM samples collected weekly for one month
  - Stored in -80° C freezer until filter extraction

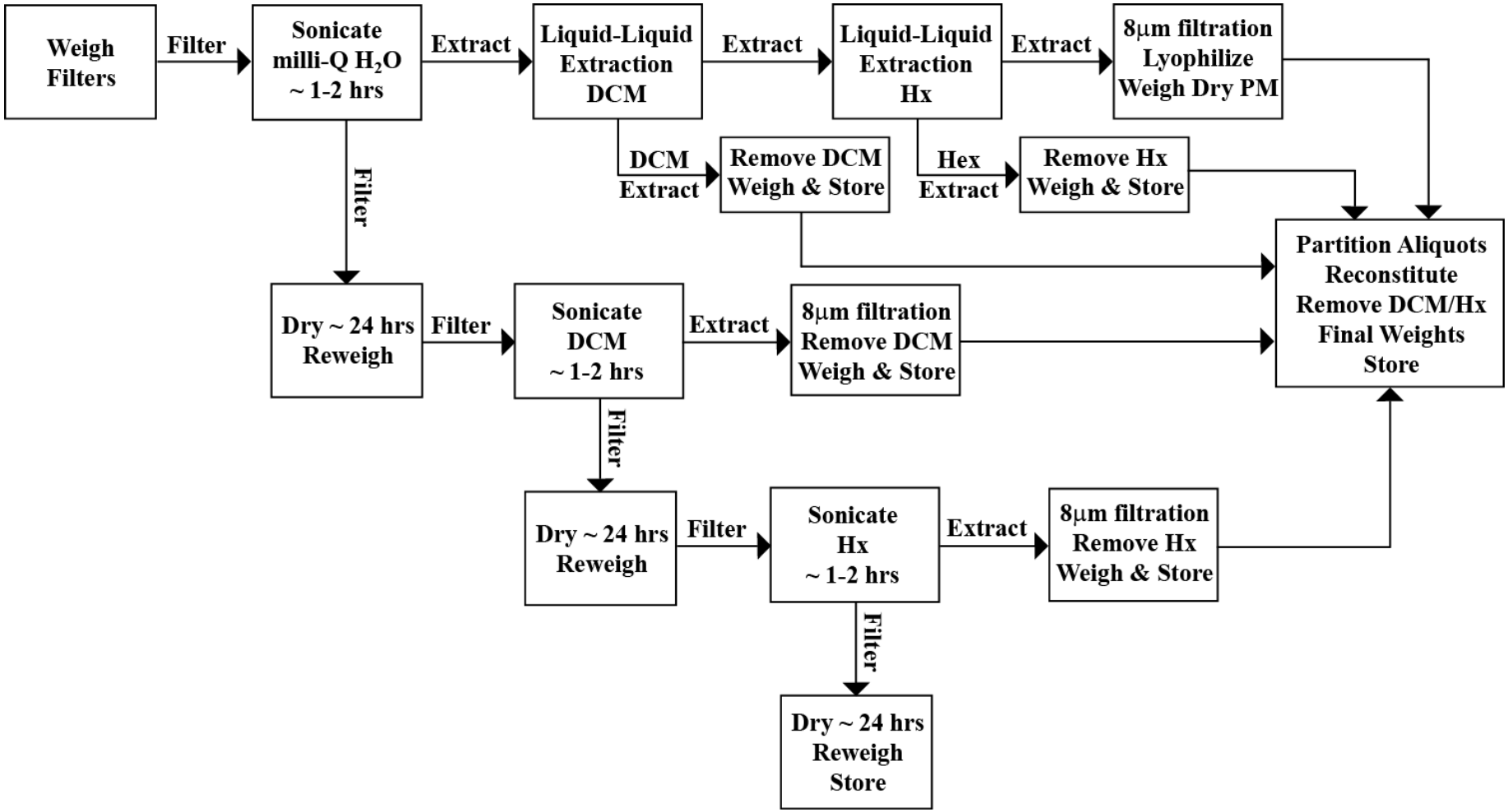
Filter Extraction Techniques

- Problem
  - PM extracted from filters for toxicological testing
  - Several filter extraction techniques available
  - Different labs employ different techniques
  - Technique employed may affect toxicological outcomes
- Solution
  - Design pilot study to test filter extraction techniques
    - Phase 1
      - Select five different filter extraction techniques
        - Sonication/Lyophilization (EPA method)
        - Multi-Solvent Extraction (MSE; Bein Lab)
        - Spin Down Extraction (SDE; Wilson Lab)
      - Prescreen extracted PM via qPCR analysis of THP-1 monocyte cell line
        - Six panel assay: IL-1b, IL-4, IL-8, GM-CSF, CYP1A1 and COX-2
      - Select top two techniques eliciting most robust response relative to control
        - MSE and SDE selected
    - Phase 2
      - Detailed compositional and toxicological inter-comparison of MSE and SDE
        - In vivo dose-response studies
      - Choose best technique for time-lag studies

Filter Extraction Techniques

Multi-Solvent Extraction (MSE)

Teflon Coated Glass Microfiber Filters



Filter Extraction Techniques
Spin Down Extraction (SDE)
Teflon Coated Glass Microfiber Filters

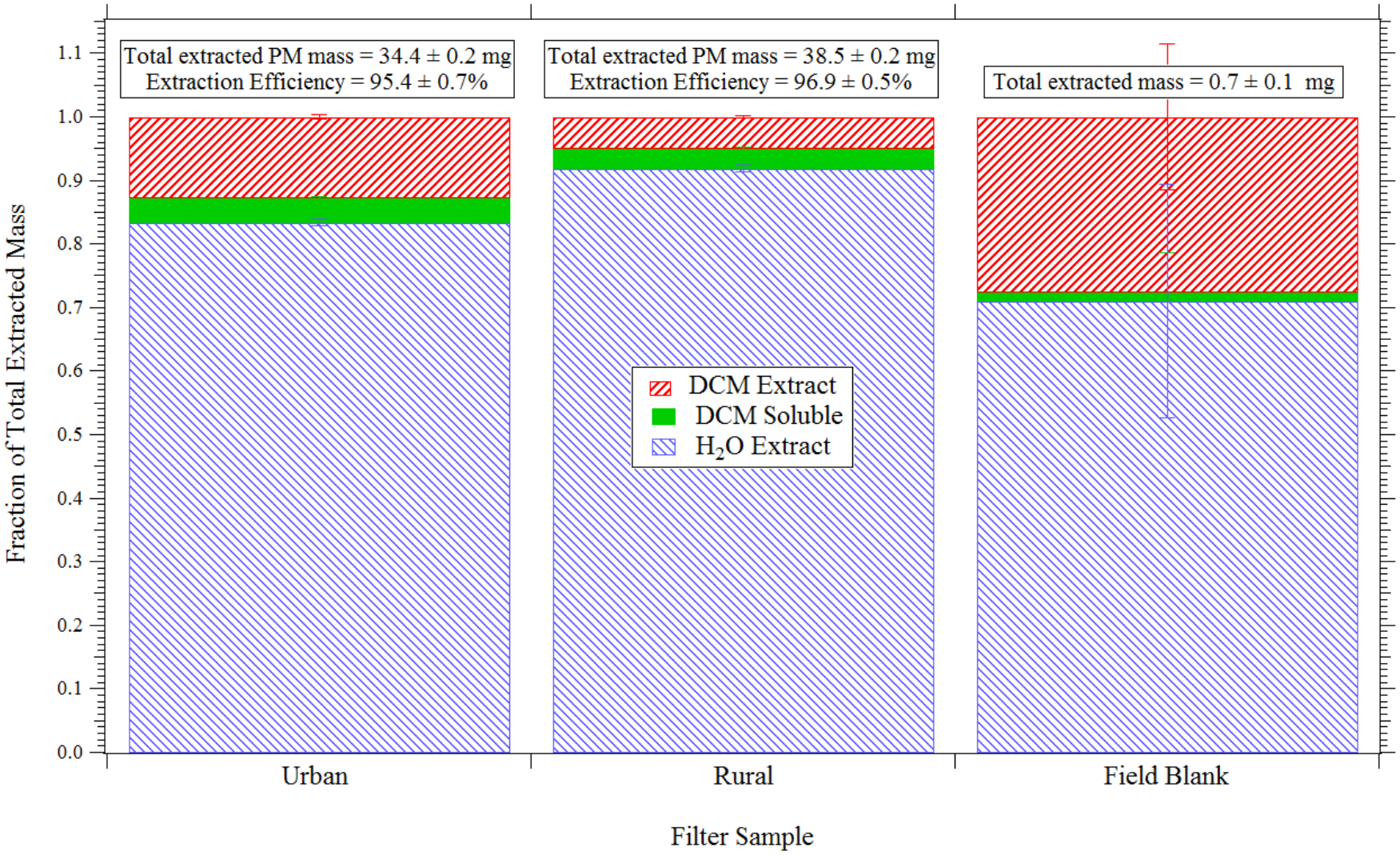
- Top layer of filter membrane with PM deposit removed, leaving filter backing behind
- Filter membranes added to top of QIAshredder® column; weighed to obtain pre-weight
- 500 µL Dulbecco's PBS without $CaCl_2$ or $MgCl_2$ added to column
- Filter membranes probe sonicated for 5 seconds
- Collection tubes attached to column and centrifuged at 7,600 x g for 4 min
- Supernatant collected from tubes and transferred back to column
- Membranes sonicated in supernatant and then centrifuged; process repeated twice
- Final centrifuged PM sample resuspended in supernatant and filtered through clean column
- Supernatant lost during process replaced with fresh PBS to obtain 500 mL final volume
- Extracted membranes in original column washed with 500 mL distilled H_2O and centrifuged
- Extracted membranes and column dried in SpeedVac concentrator for 6 hours
- Extracted membranes and column weighed to obtain extraction post-weight
- Extraction pre- and post-weights subtracted to obtain extracted PM mass

Filter Extraction Techniques
MSE versus SDE

- Multi-Solvent Extraction Objectives
  - Designed for source-oriented sampling studies
    - Bein et al. *Atmospheric Environment* 90: 87-95, 2014.
  - Maximize extraction efficiency (> 95%)
  - Minimize compositional biases
  - Minimize extraction artifacts
    - Filter glass microfibers (FGMs) unavoidably present in extract
    - Selective filtration removes ~ 60-70% by mass
- Spin-Down Extraction Objectives
  - Maximize FGM removal efficiencies
  - Avoid organic solvents
- Key Differences
  - Extraction solvents
    - MSE: H_2O, DCM and Hx followed by solvent removal
    - SDE: sonication directly into PBS delivery vehicle
  - Post-extraction cleanup
    - MSE: microporous membrane filtration
    - SDE: centrifugal homogenization and filtration
  - Gravimetric analysis
    - MSE: direct measurement of extracted PM mass
    - SDE: difference between pre- and post-extracted filter mass

Multi-Solvent Extraction

Fractional Distribution of Total Extracted PM Mass

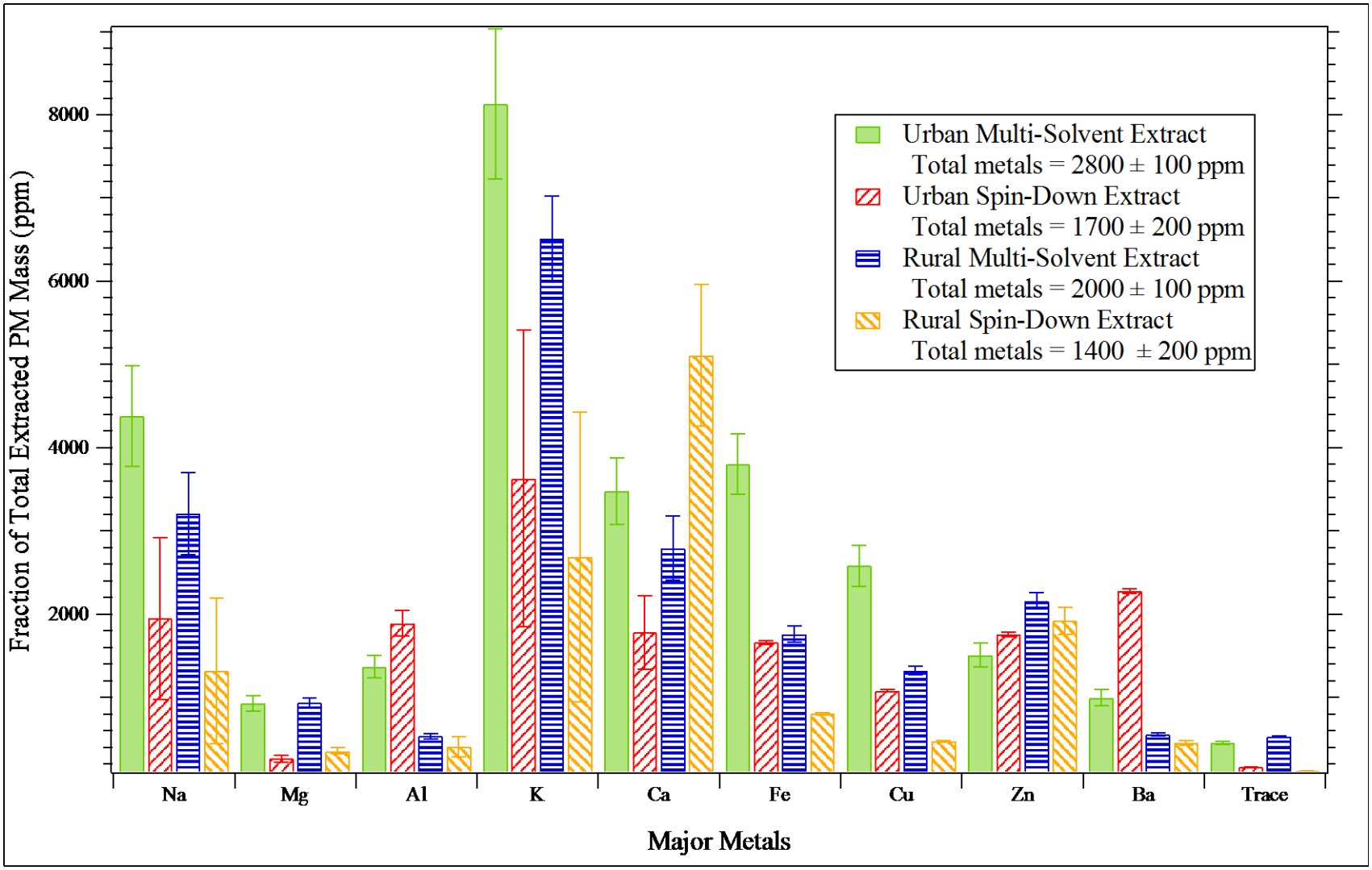


Chemical Characterization

- Novelties
  - Sample preparation techniques
  - Exhaustive chemical characterization of single PM extract
  - Analyze same PM and field blank extracts as used in exposure studies
  - Comprehensive compositional inter-comparison of filter extraction techniques
- Trace Metals
  - Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)
    - Li, Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Tl, Pb, U
  - Sample Preparation (novel method)
    - Liquid-liquid extraction (DCM/Hx)→ Acid digestion (HNO_3) →Sonication
- Water Soluble Inorganic and Organic Ions
  - Ion Chromatography; Automated Colorimetry; Atomic Absorption Spectrophotometry
    - NH_4^+, Cl^-, NO_2^-, NO_3^-, SO_4^{-2}, PO_4^{-3}, Na^+, Mg^{+2}, K^+, Ca^{+2}, 17organic sugars, 9 organic acids
  - Sample Preparation
    - Dilution → Sonication → 0.2 μm filtration
- Molecular Organic Compounds
  - Thermal Desorption-Gas Chromatography Mass Spectrometry (TD-GCMS)
    - 38 PAHs, 47 high molecular weight alkanes/alkenes, 18 hopanes, 12 steranes
  - Sample Preparation (novel method)
    - MeOH Sonication → MeOH drip onto pure quartz filters under reverse N_2 flow
    - Average mass transfer efficiency = 97 ± 8%
- Elemental Carbon and Organic Carbon (EC/OC)
  - Thermal Optical Reflectance (TOR)
  - Sample Preparation (same as Molecular Organic Compounds)
- Corrections and Error Estimates
  - All PM composition data field blank and process blank corrected
  - Measurement errors propagated through all calculations to obtain 99% confidence intervals

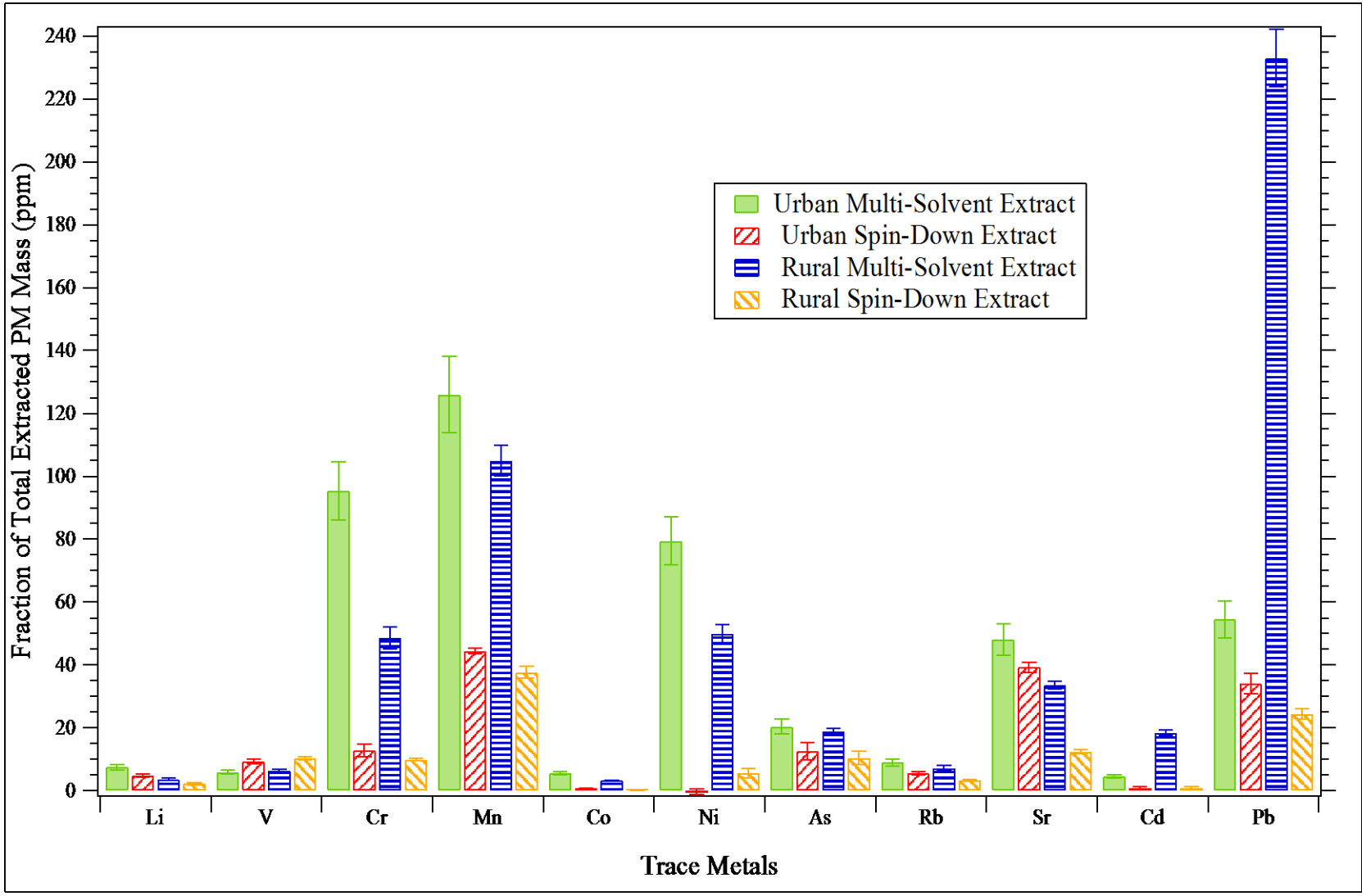
Comparative Compositional Analyses

Mass Fraction Data – Major Metals



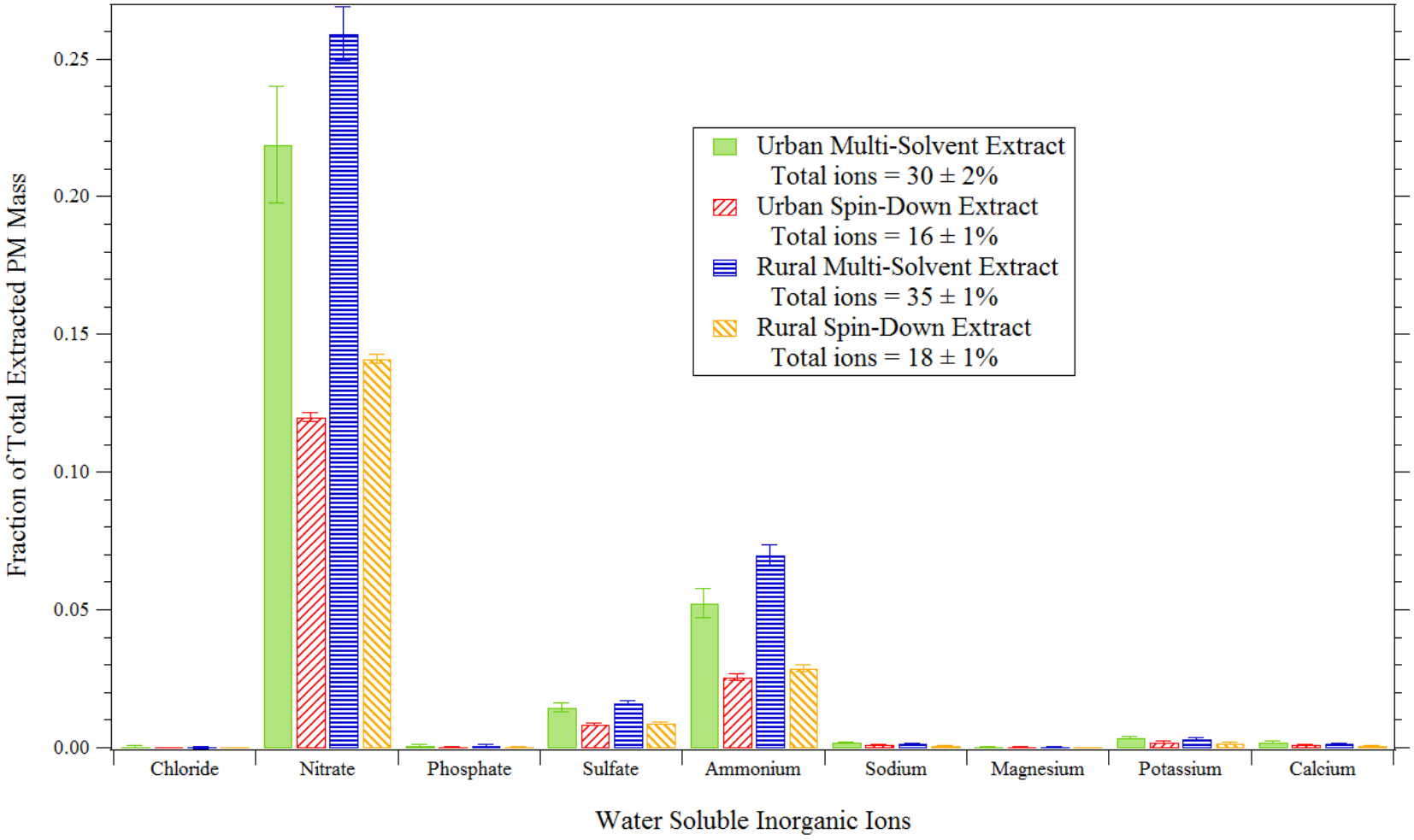
Comparative Compositional Analyses

Mass Fraction Data – Trace Metals



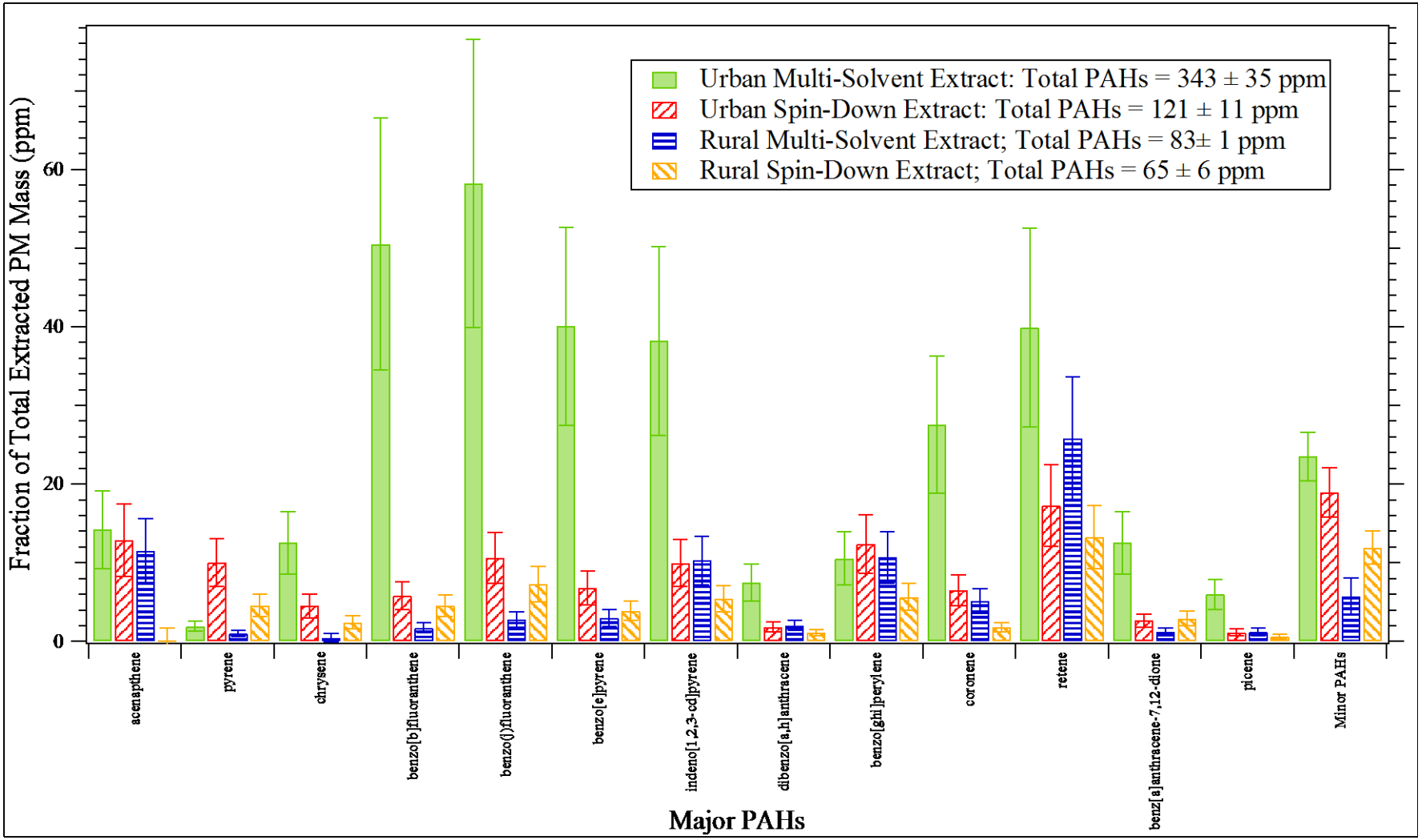
Comparative Compositional Analyses

Mass Fraction Data – Water Soluble Inorganic Ions



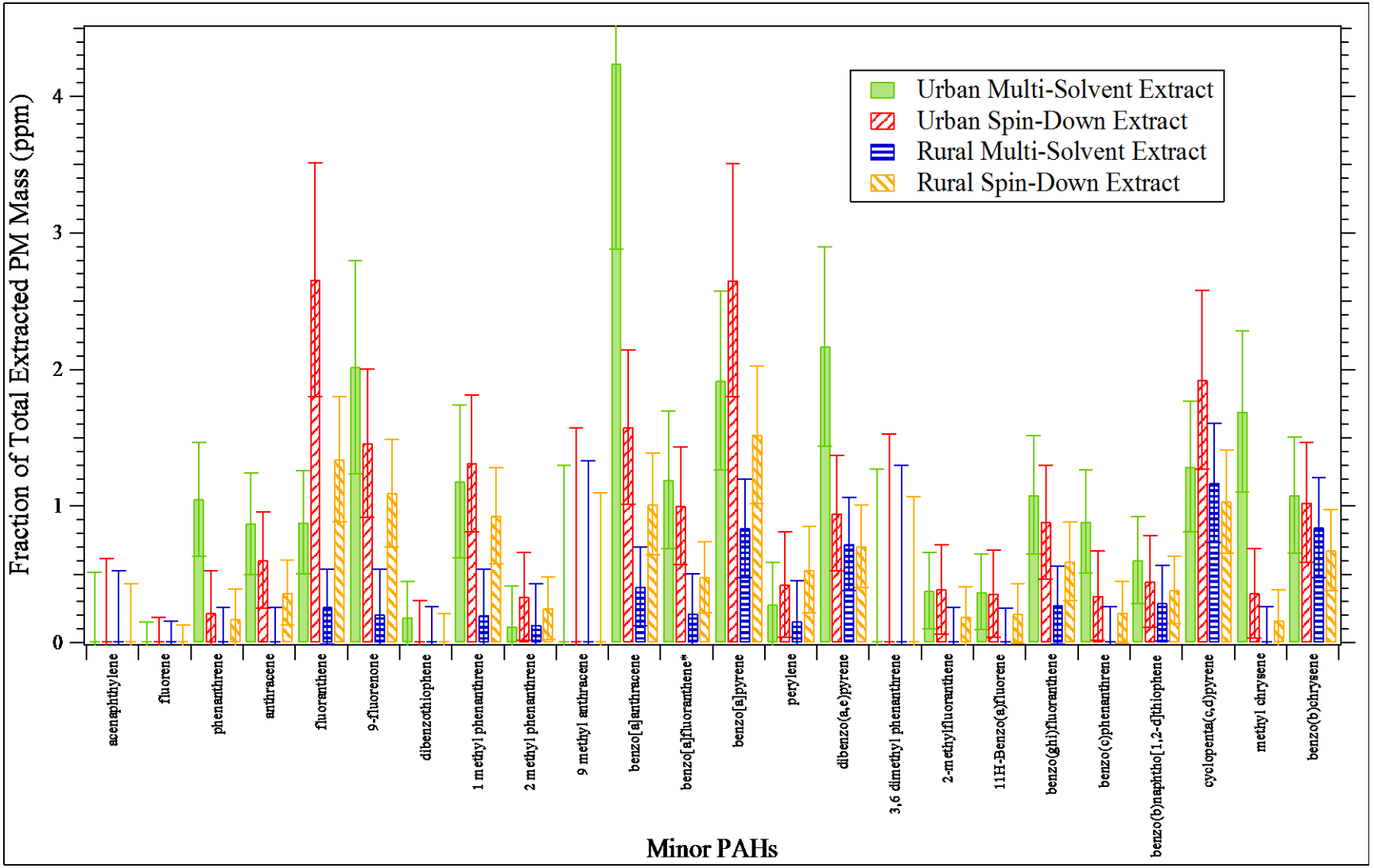
Comparative Compositional Analyses

Mass Fraction Data – Major PAHs



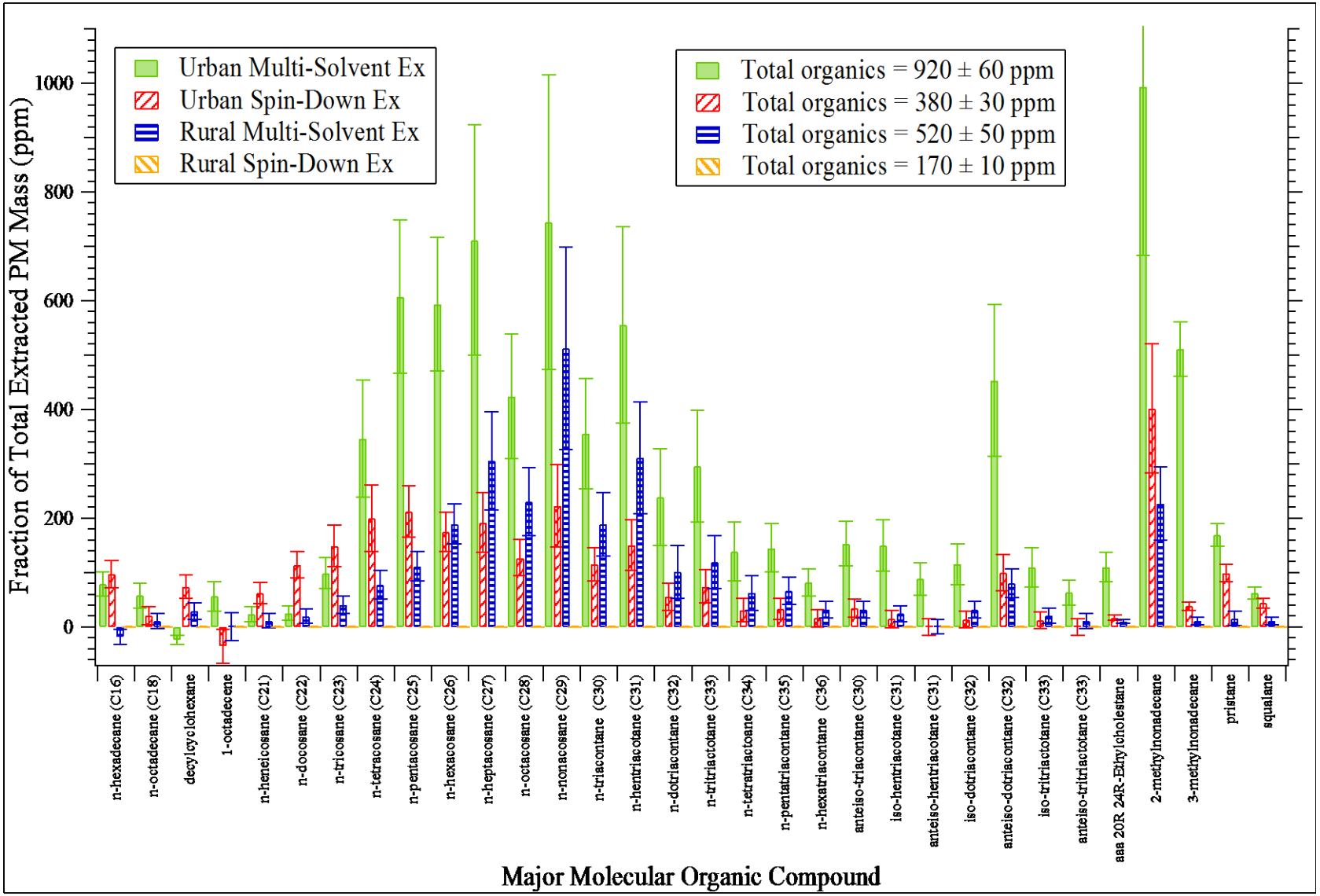
Comparative Compositional Analyses

Mass Fraction Data – Minor PAHs



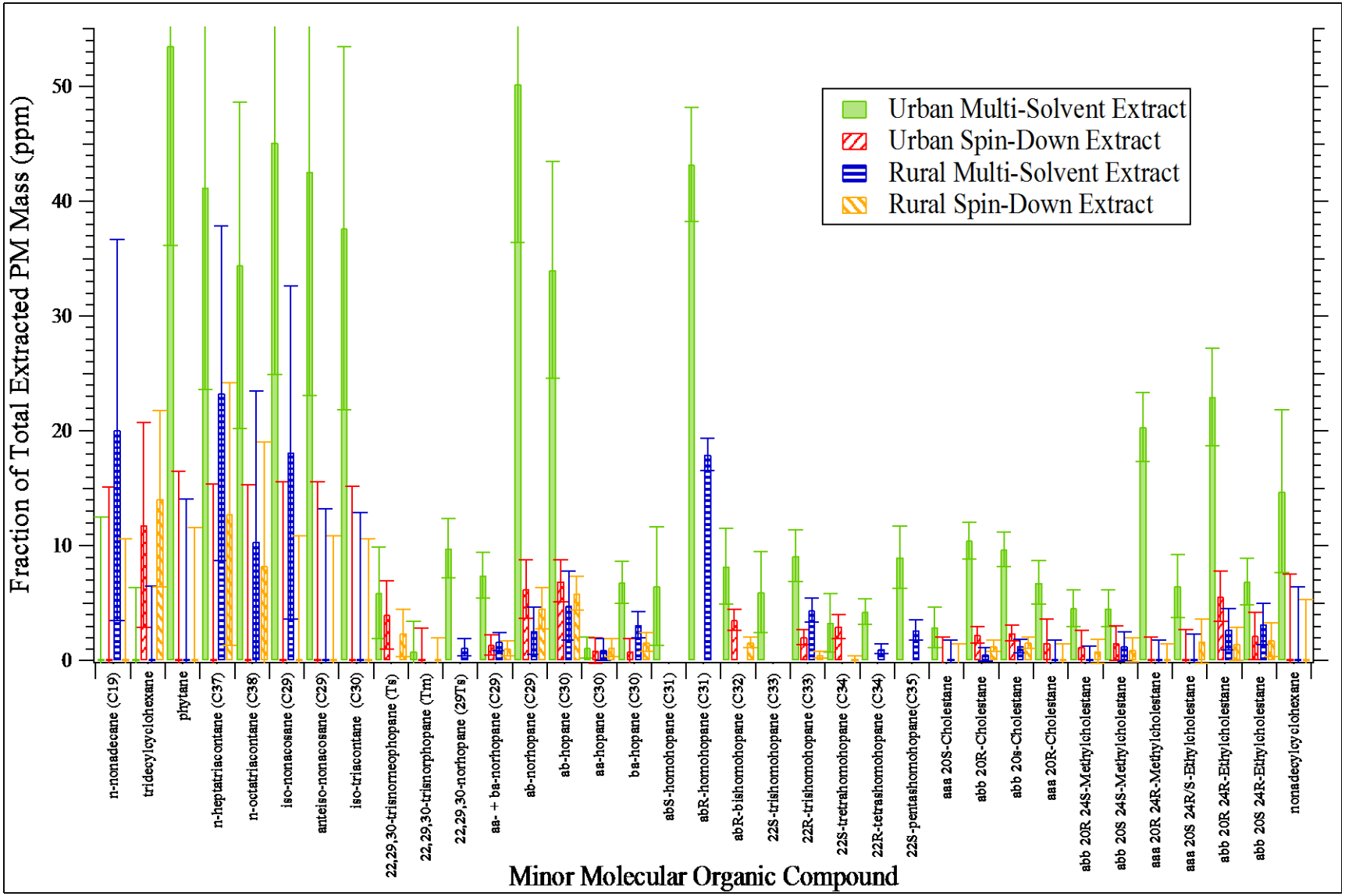
Comparative Compositional Analyses

Mass Fraction Data – Major Non-Aromatic Organics



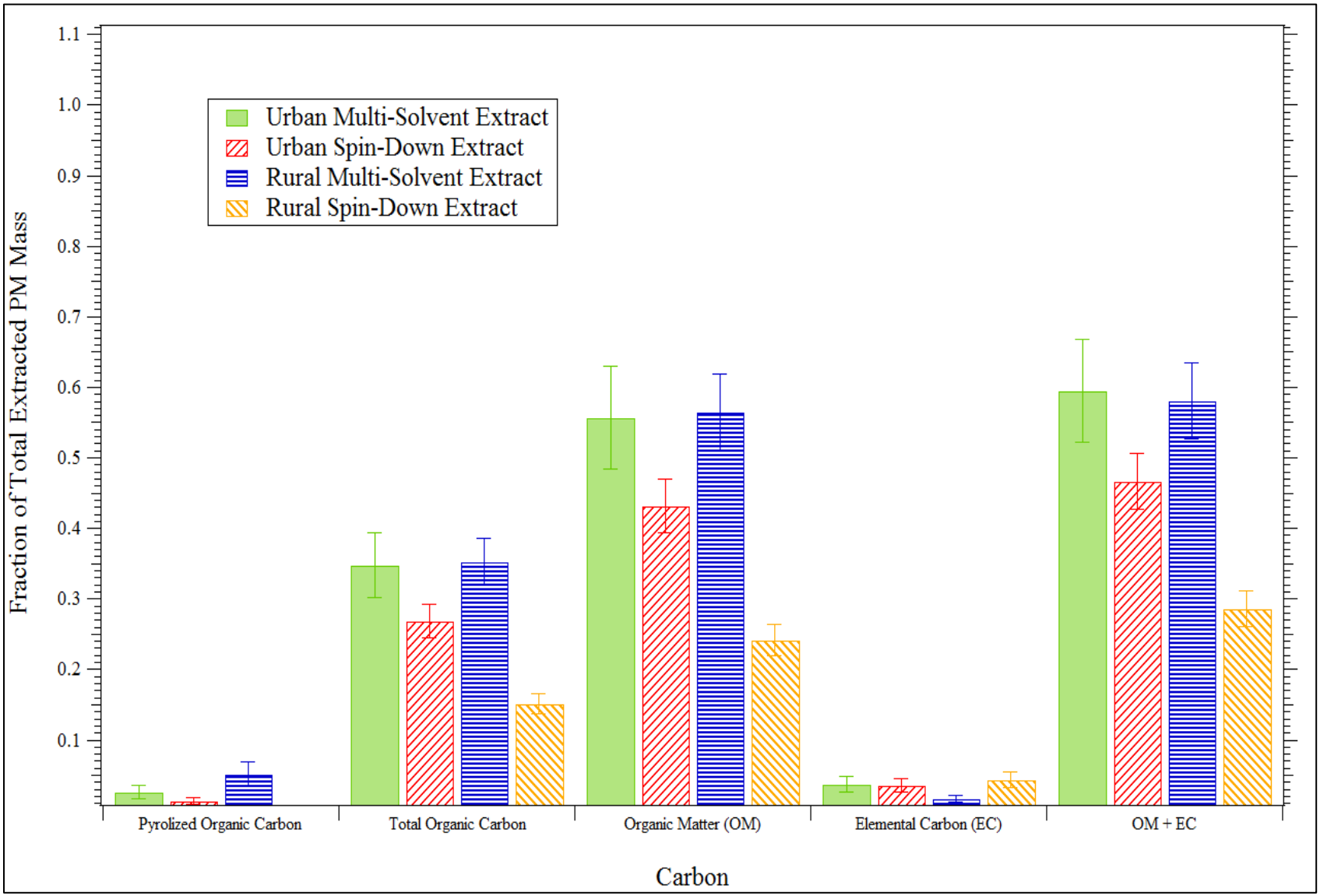
Comparative Compositional Analyses

Mass Fraction Data – Minor Non-Aromatic Organics



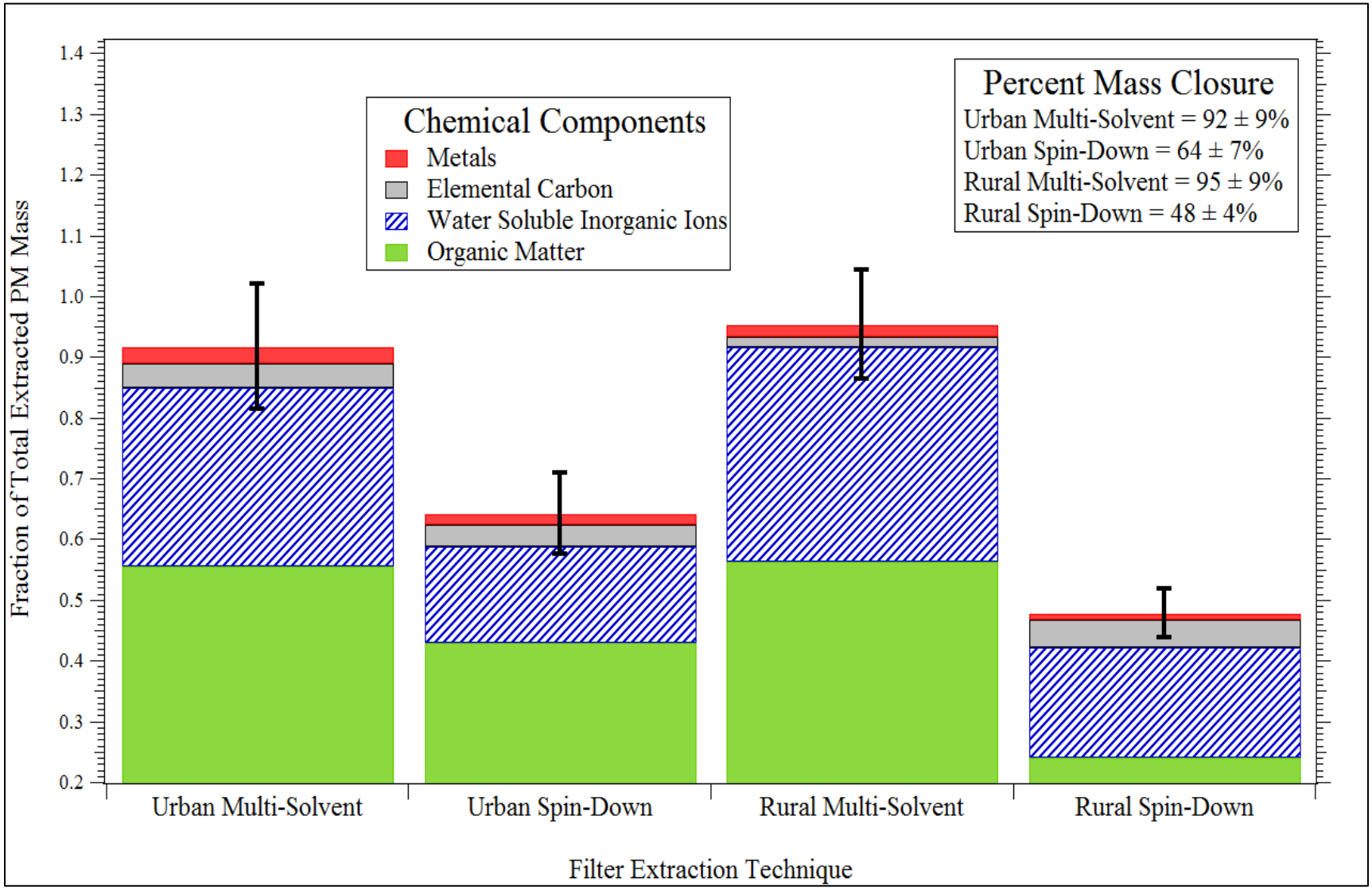
Comparative Compositional Analyses

Mass Fraction Data – EC/OC



Comparative Compositional Analyses

Mass Closure

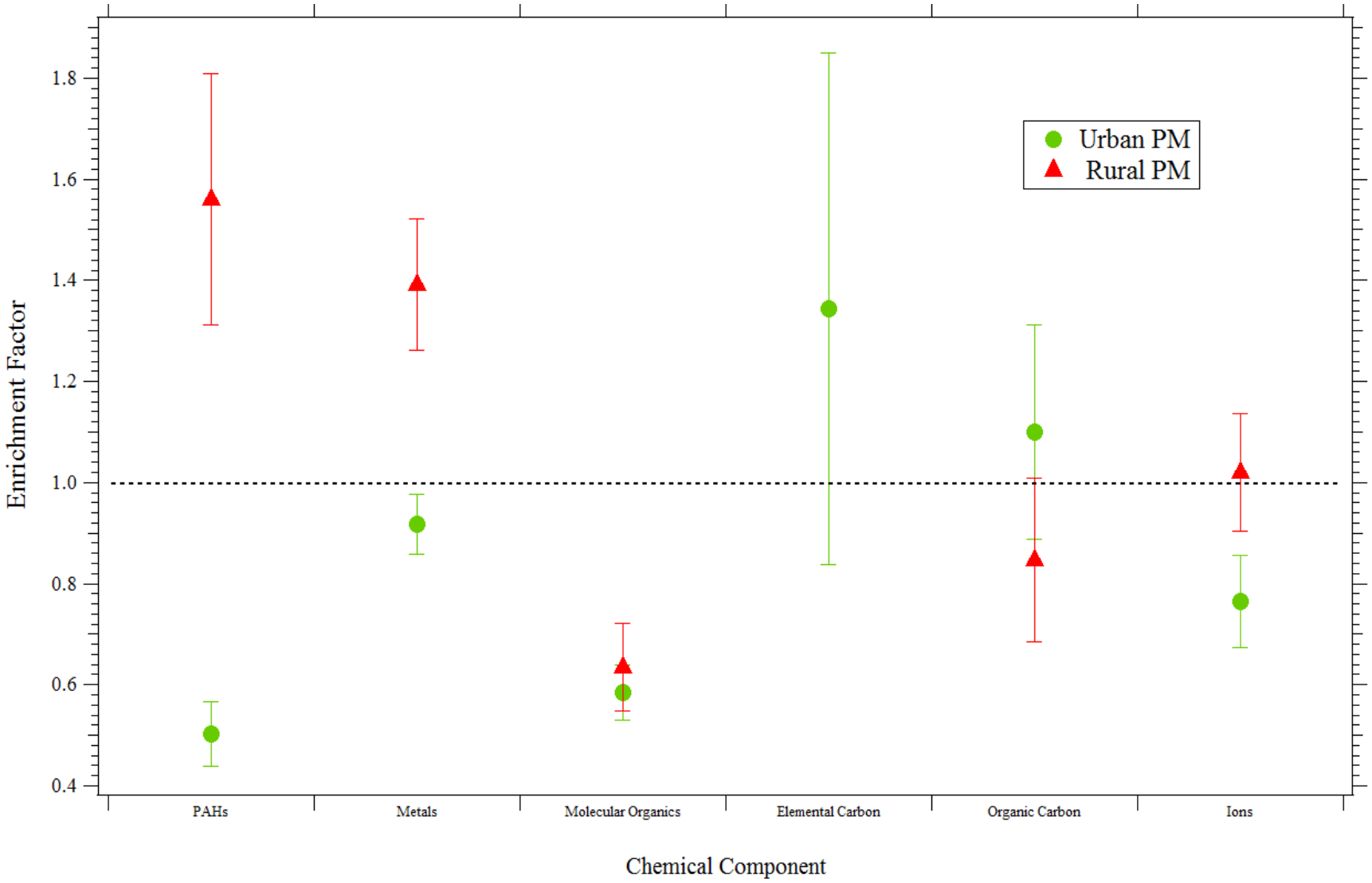


Comparative Compositional Analyses
Retrospective Mass Reconciliation

- Mass Closure
  - Large fraction of SDE extracted PM mass unaccounted for: urban = 36 ± 7%; rural =52 ± 4%
  - MSE extracts well characterized by measured chemical components
  - SDE method never directly measures total extracted PM mass
  - Hypothesis: unaccounted PM mass lost in the SDE process
- Retrospective Mass Reconciliation
  - Primary issue
    - SDE extracts directly into PBS so high salt concentrations
      - 5.28 mg buffering salts compared to ~ 4-5 mg PM per extract
      - Potentially large measurement errors in directly measuring PM mass
  - Approach
    - Compare SDE filter blank extract mass to volume-calculated PBS salt mass
    - Use volume-calculated PBS salt mass to correct SDE PM extract mass
    - Propagate error between filter blank and volume-calculated mass to quantify uncertainties
  - Methods
    - Archived aliquot of SDE filter blank and PM extract apportioned evenly among six new aliquots
    - Blow down MeOH and residual PBS H_2O content under N_2 atmosphere
    - Directly weigh dried extracts via analytical microbalance; calculate average ± 99% confidence interval
  - Results
    - Percent difference between weighed filter extracts and volume-calculated mass = 0 ± 6%
    - PBS-adjusted PM extract masses show significant mass lost during SDE process
      - Urban = 44 ± 9%; rural = 52 ± 8%
    - Adjusted masses significantly improve SDE mass closure
      - Urban: 64 ± 4% → 110 ± 13%; rural: 48 ± 3% → 100 ± 10%

Comparative Compositional Analyses

Adjusted SDE-to-MSE Mass Enrichment Factors



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Animal Exposure Studies

- Blind studies performed prior to chemical characterization
- Dose-Response Study
  - Characterize dose response to filter blank and urban extracts
  - Use MSE and SDE extracts to inter-compare techniques
  - Select extraction technique eliciting most robust response
  - Select moderate response dose for urban-rural comparison
- Time-Delay Study
  - Characterize temporal response to single acute exposure
  - Use extract and dose selected from Dose-Response Study
  - Use urban and rural extracts to inter-compare environments
  - Include both pulmonary and vascular systems

Animal Exposure Methods

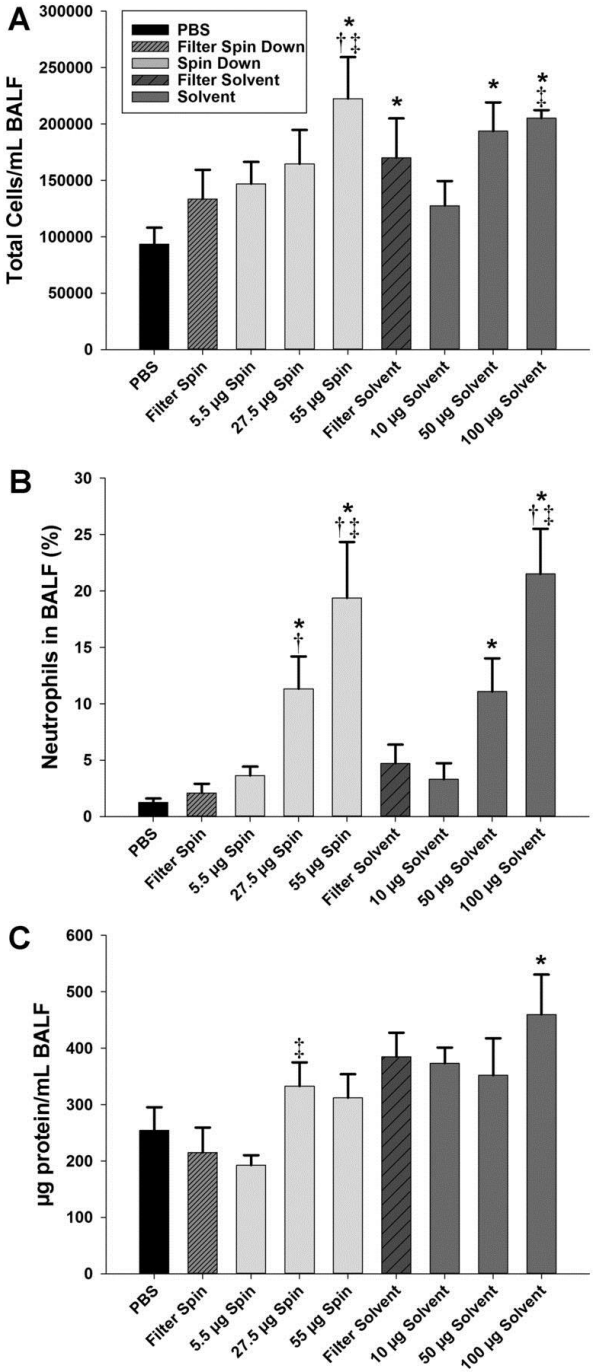
- For all exposures
  - PM and filter blank extracts resuspended in PBS
  - Resuspended extracts sonicated for ~ 5 minutes
  - Sonicated extracts administered via oropharyngeal aspiration
  - Eight week reproductively capable adult male BALB/c mice
    - Acclimated in filtered air for 7 days
    - All animal procedures followed IACUC protocols
  - Control mice: 50 μL suspensions of filter blank extracts
  - Treated mice: 50 μL suspensions of PM extracts
  - Six mice used for each time point and exposure dose
  - Animals euthanized via intraperitoneal injection of pentobarbital
  - At necropsy:
    - Tracheas cannulated, thorax opened and lung removed

Toxicological Assay Methods

- BALF Differentials and Total Protein
  - Right lung lobe only
  - Total cells, % neutrophils and total protein
  - All samples assayed in triplicate
- Quantitative RT-PCR
  - RNA isolated from microdissected intrapulmonary airways and surrounding parenchymal tissue
  - Dose-response: CYP1A1, CYP1B1 and IL-1B
  - Time-delay: 26 different genes analyzed
- Immunohistochemistry
  - Left lung lobe paraffin sections of 3 mice per treatment group
  - Rabbit anti CYP1A1 and CYP1B1 antibodies
  - Detect primary antibody binding sites
- Histologic Evaluation
  - Two lung sections representing short and long axial pathways of left lung lobe
  - All terminal bronchiole-alveolar duct junctions evaluated
  - Each section assigned an overall severity score
- Platelet Alpha Granule Proteins and Integrins
  - Platelet activation analyzed in whole body via flow cytometry
- Bioplex Analysis of Lung and Serum Cytokines
  - Time-delay study only
  - Protein assays performed on subset of 3 animals per time point and treatment
  - Total of 32 cytokines assayed
- Statistics
  - All data reported as mean ± standard error of the mean
  - Statistical outliers eliminated via extreme studentized deviate method
  - Intra-treatment group comparisons for continuous data performed via one-way ANOVA followed by PLSD
  - Lesion scoring analyzed via Kruskal-Wallis one-way ANOVA test; p values < 0.05 statistically significant

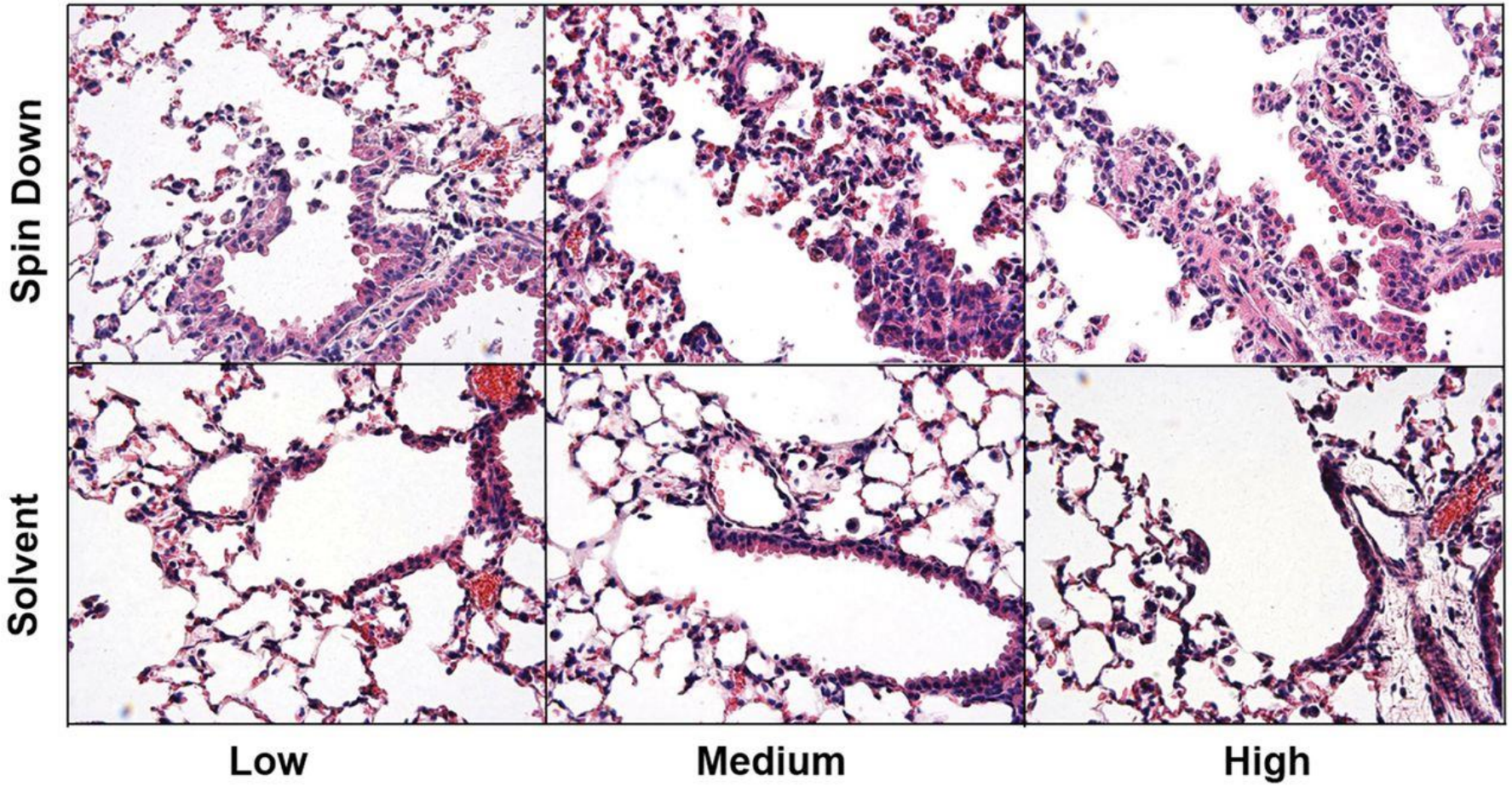
Dose-Response Study (MSE vs SDE)

- Blind study
  - MSE and SDE urban PM extracts
  - 10, 50, 100 μg doses
- SDE Dosing Problem
  - Retrospective mass reconciliation
    - PM mass lost during SDE process
    - Dosing masses not equivalent
      - SDE dose ~ ½ that of MSE dose
    - SDE doses corrected
      - 5.5, 27.5 and 55 μg
- BALF analyzed for
  - Total cellular infiltrates (A)
  - Percent neutrophils (B)
  - Total protein (C)



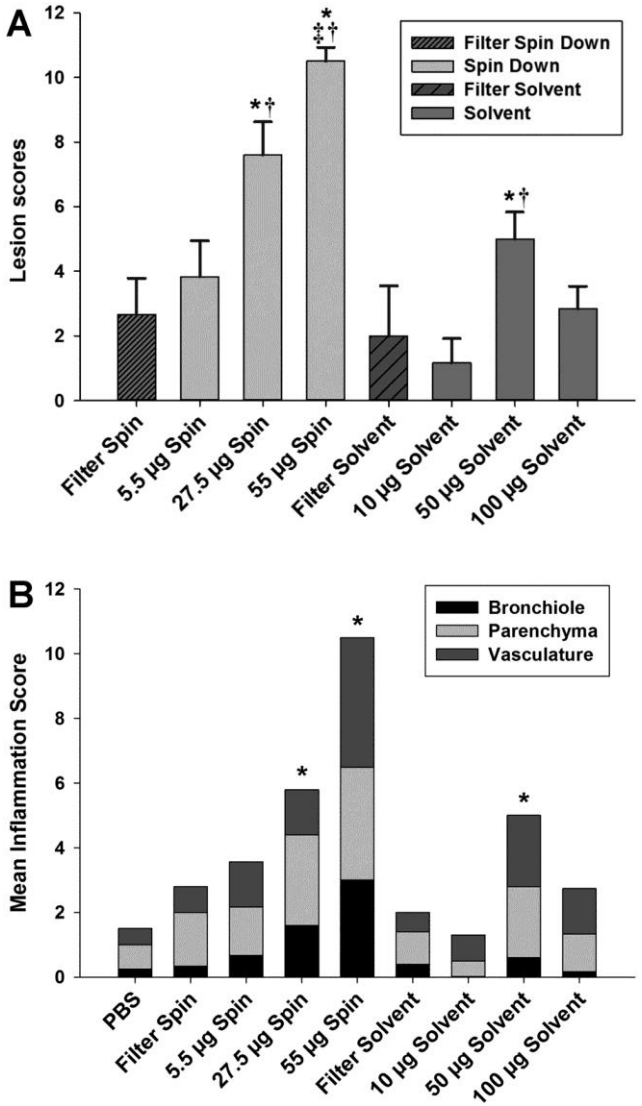
Dose-Response Study (MSE vs SDE)

Lung Lesions



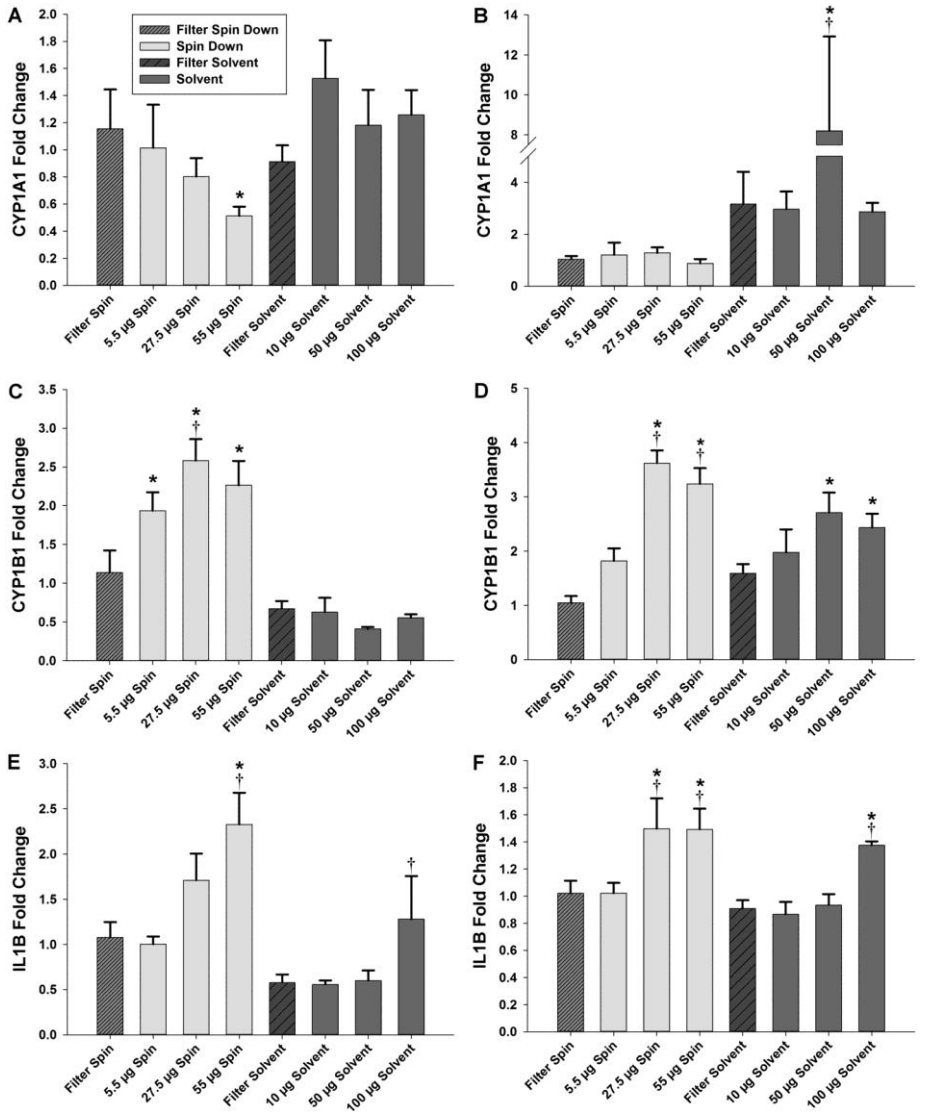
Dose-Response Study (MSE vs SDE)

Mean Inflammation and Lesion Scores



Dose-Response Study (MSE vs SDE)
CYP1A1, CYP1B1 and IL-1B Expression

Conducting Airways



Whole Lung

Dose-Response Study (MSE vs SDE)
Immunohistochemical Localization

CYP1A1 SDE filter control

CYP1A1 MSE filter control

CYP1A1 SDE PM extract

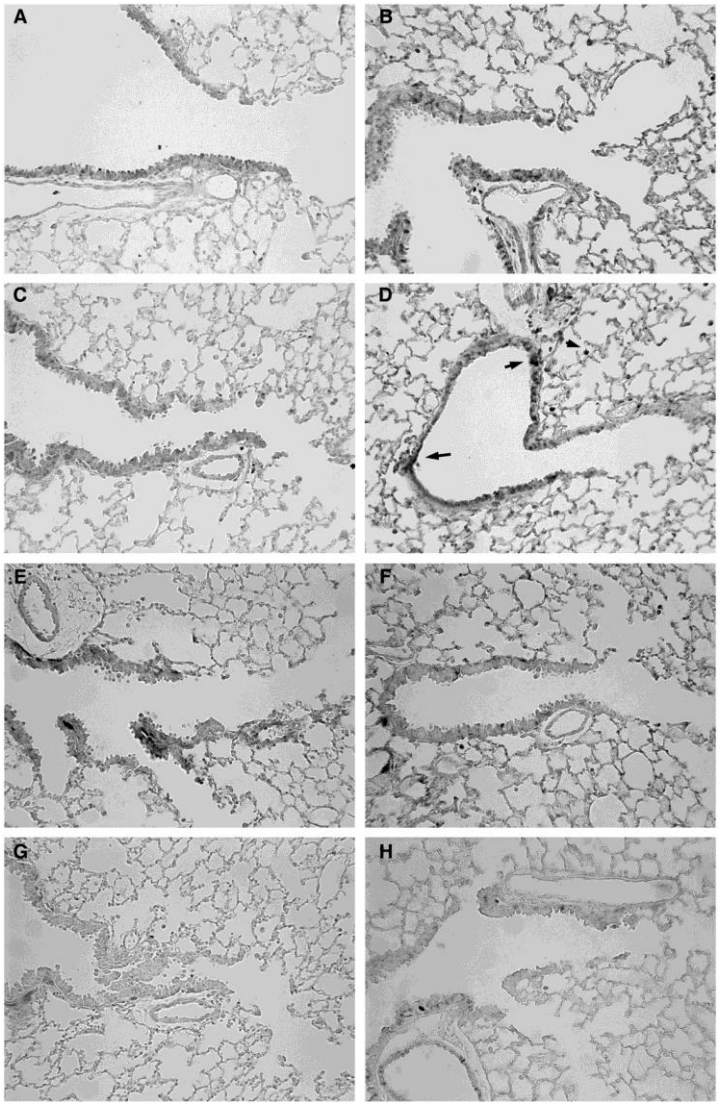
CYP1A1 MSE PM extract

CYP1B1 SDE filter control

CYP1B1 MSE filter control

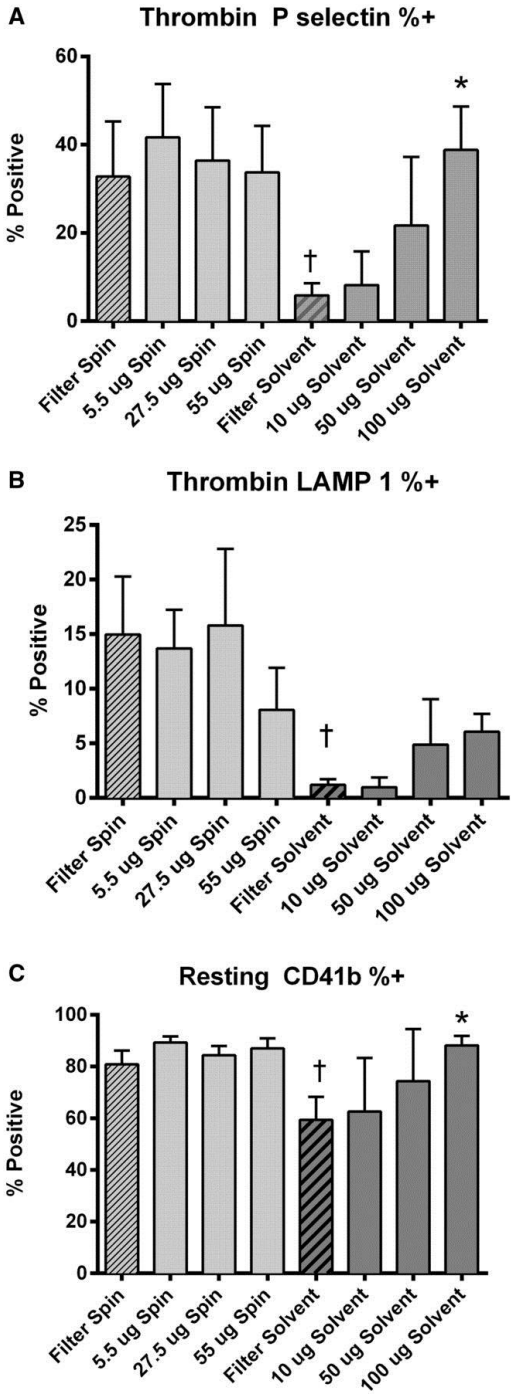
CYP1B1 SDE PM extract

CYP1B1 MSE PM extract



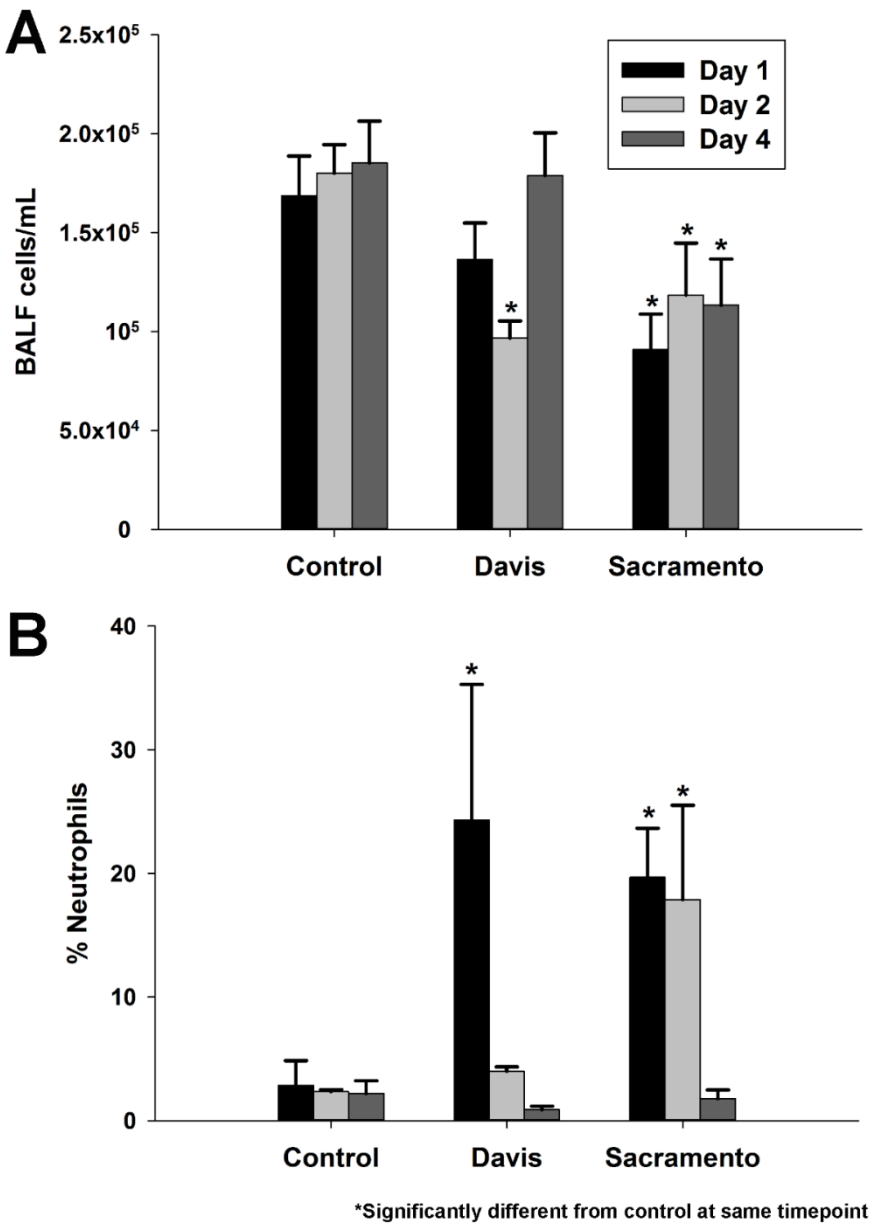
Dose-Response Study (MSE vs SDE)

- Flow cytometric analysis of expression of (A) platelet alpha granule membrane protein P-selectin, (B) platelet lysosomal granule protein (LAMP-1) in platelets activated by thrombin agonist and (C) integrin CD41b on resting platelet surface



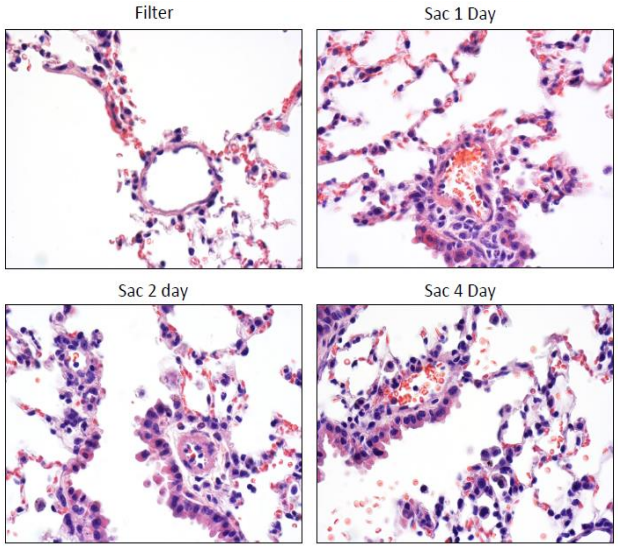
Time-Delay Study (Urban vs Rural)

- Blind Study
- Urban and Rural Extracts
- Necropsies at 1, 2 and 4 days post-exposure
- Filter blank extract controls for all time points
- From dose-response study
  - SDE extracts selected
  - Selected SDE dose = 50μg
- BALF total cells (A) and percent neutrophils (B)

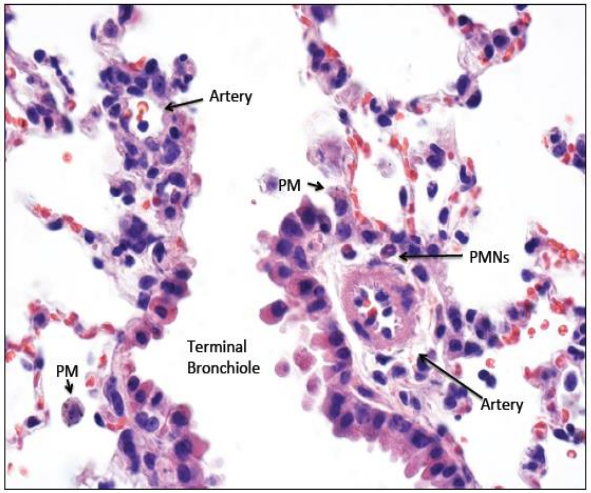


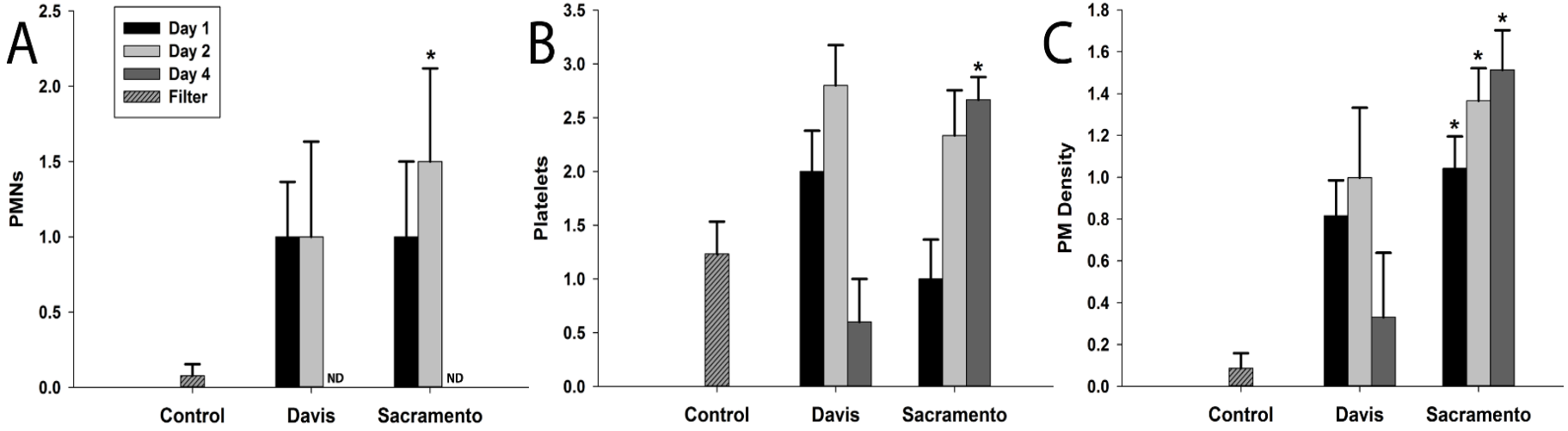
Time-Delay Study (Urban vs Rural)

Histologic Changes at Terminal Bronchiolar Junction



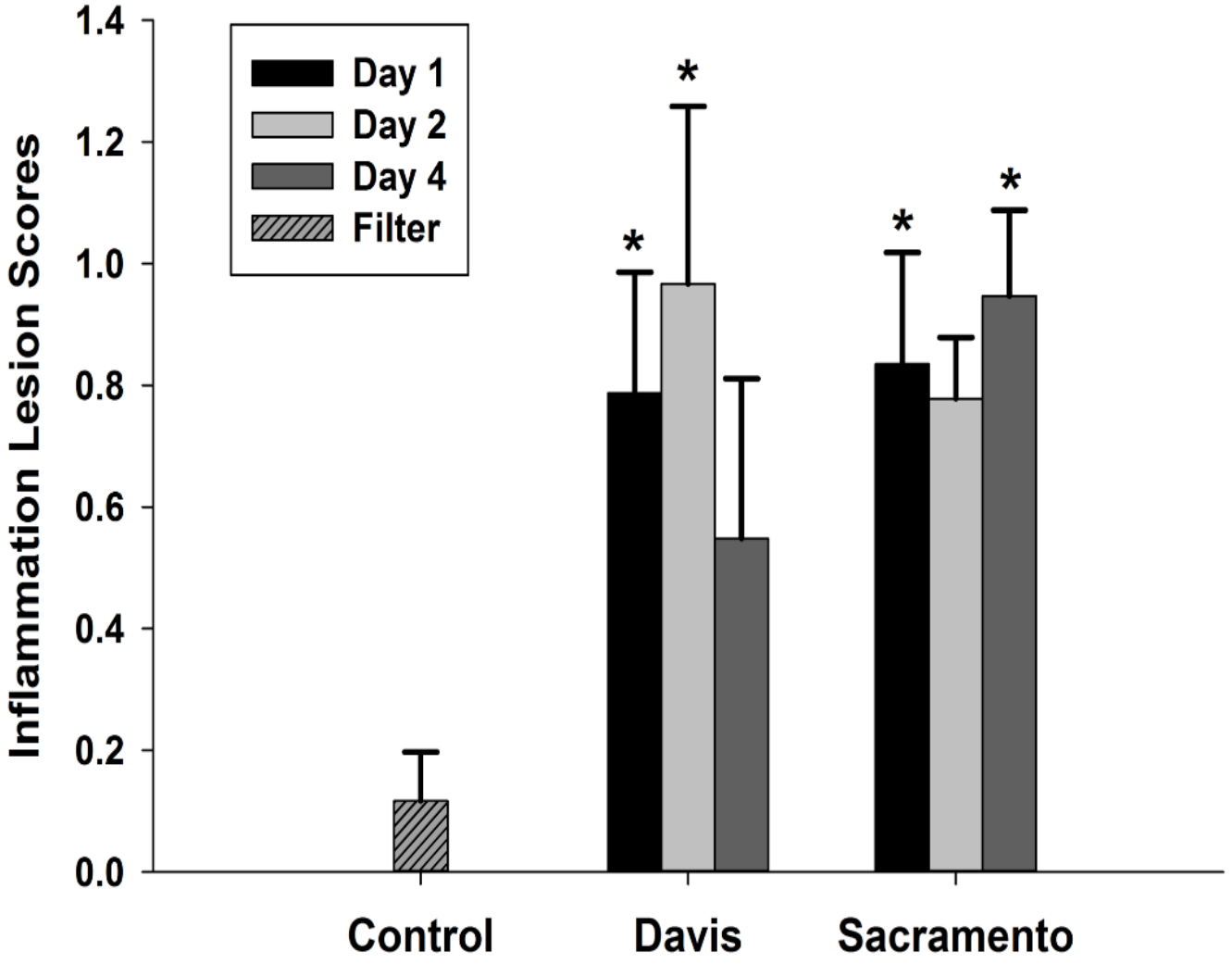
Sac 2 day magnified



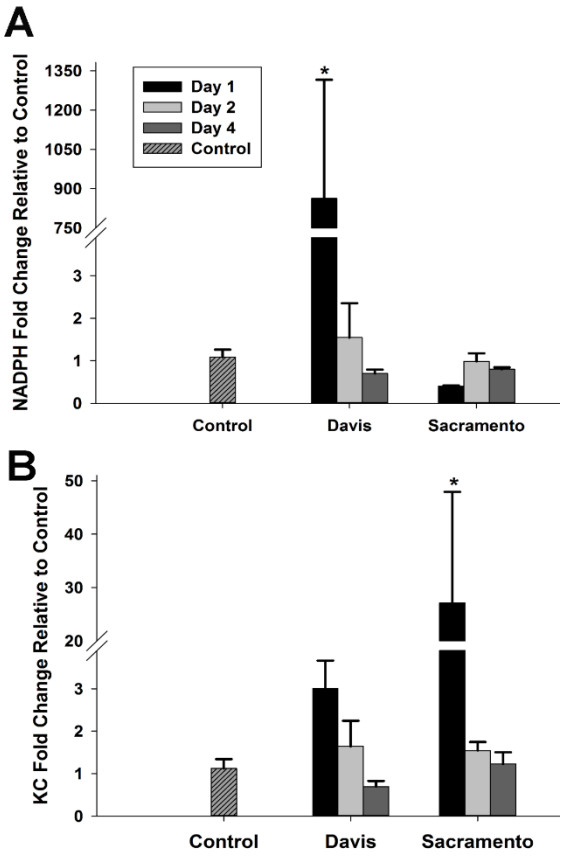


Lesion scoring by histologically defined inflammation components

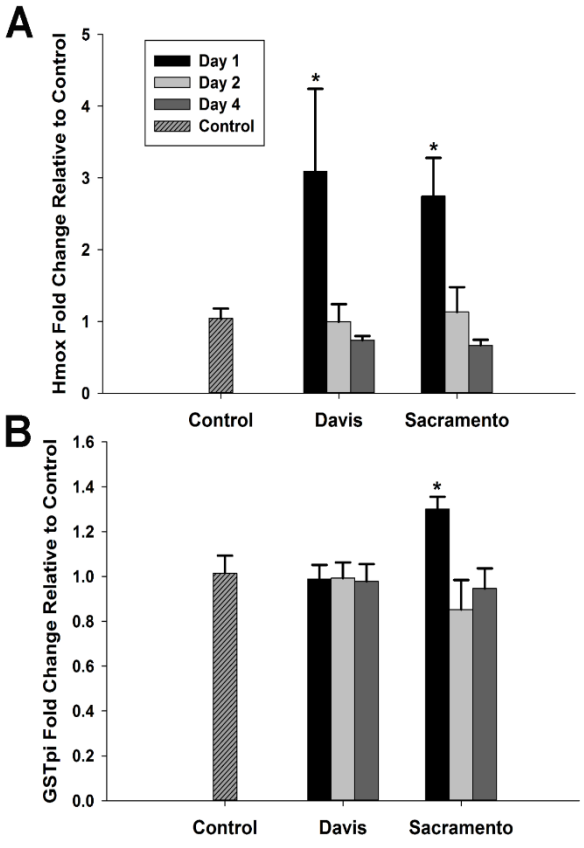
Time-Delay Study (Urban vs Rural)
Overall Lung Inflammation Lesion Scores



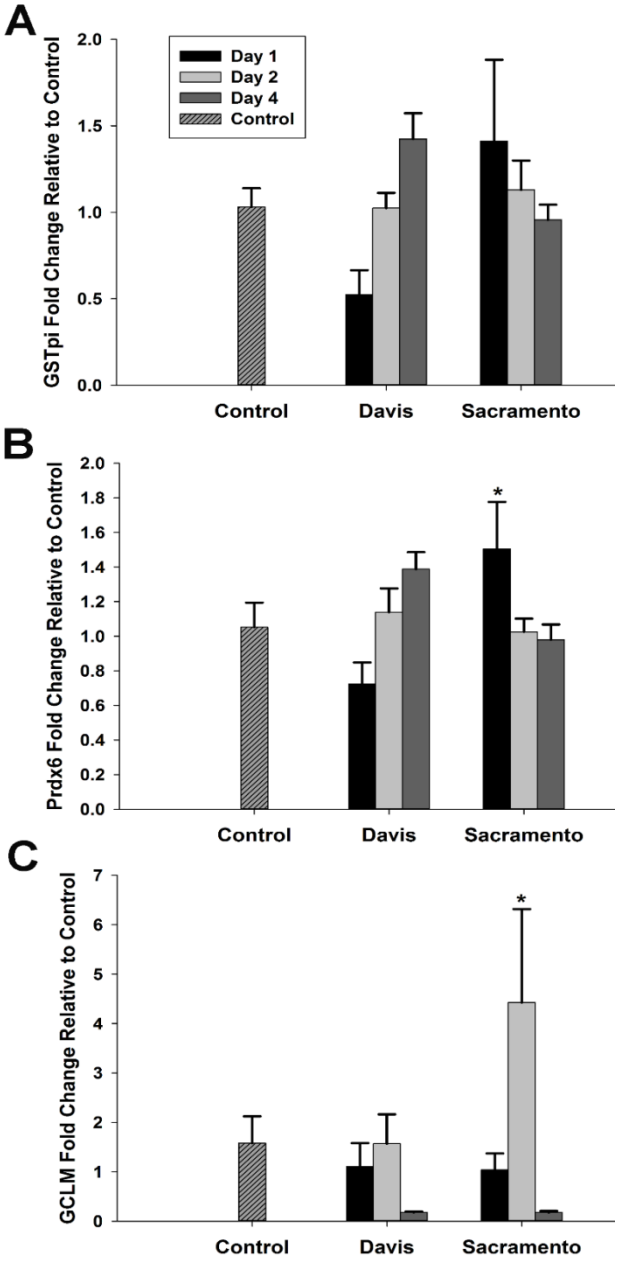
Time-Delay Study (Urban vs Rural)
Gene Expression



Expression of key genes involved in inflammatory cell recruitment to lung tissue

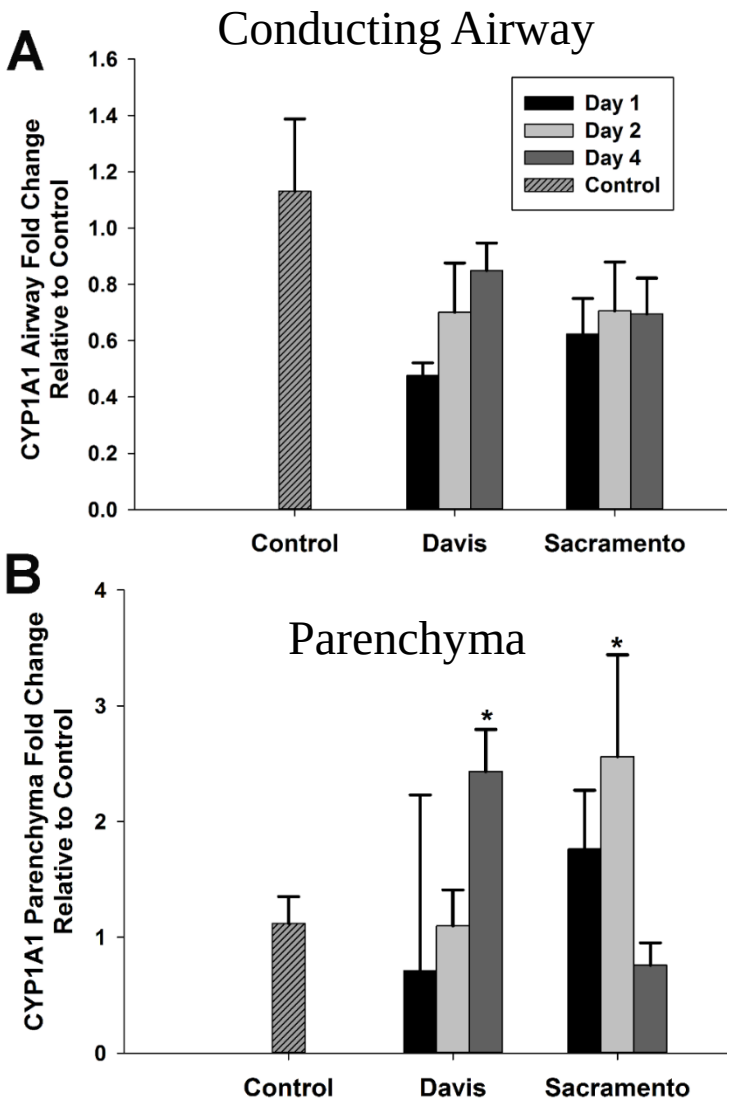


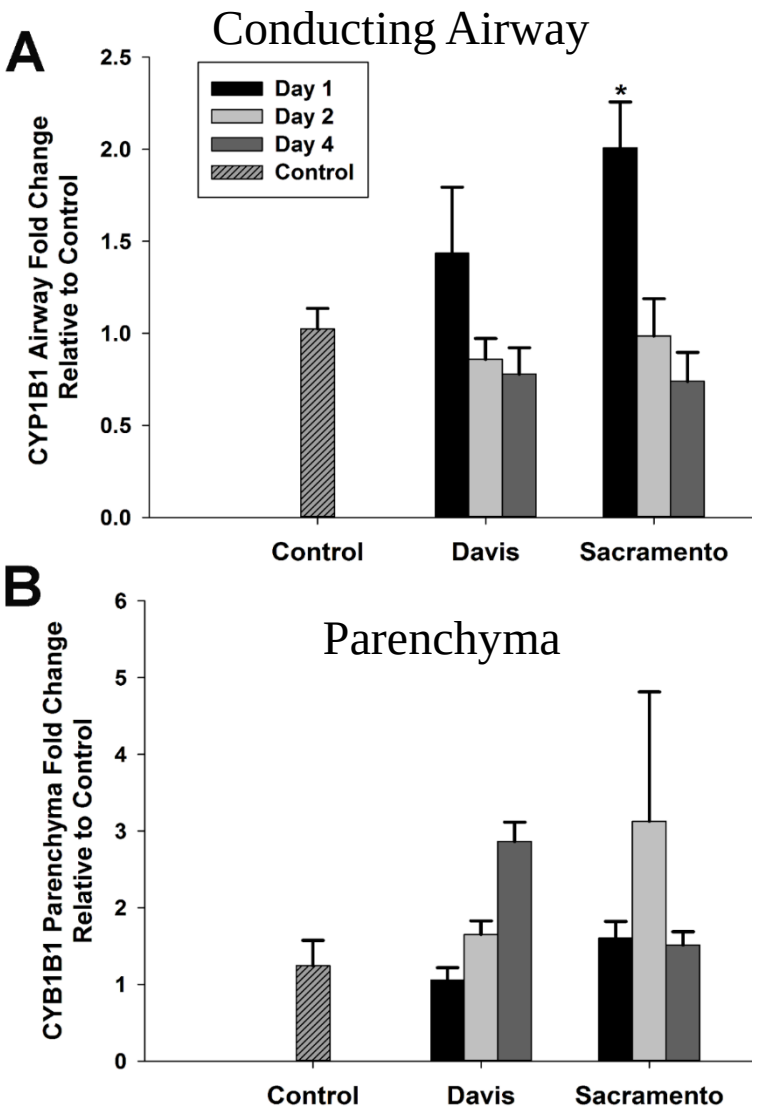
Conducting airway expression of key antioxidant and phase 2 metabolism genes



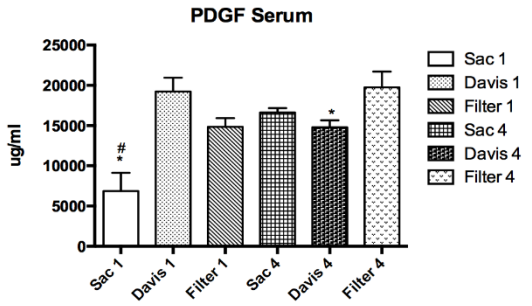
Parenchymal expression of key antioxidant and phase 2 metabolism genes

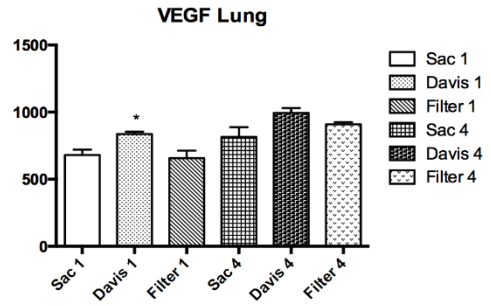
Time-Delay Study (Urban vs Rural)
CYP1A1 and CYP1B1 mRNA Expression

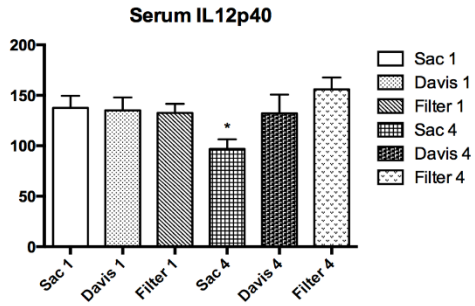


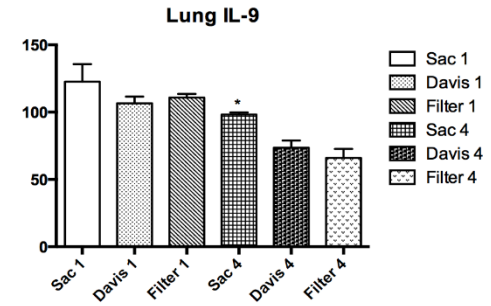


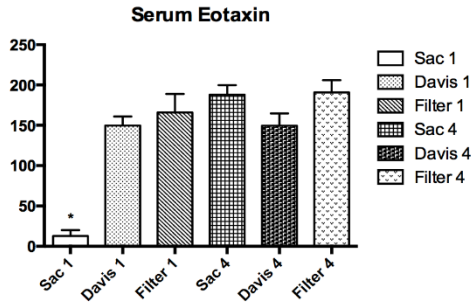
Time-Delay Study (Urban vs Rural)
Multiplex Cytokine Assays

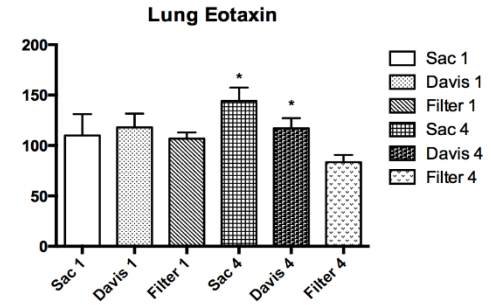








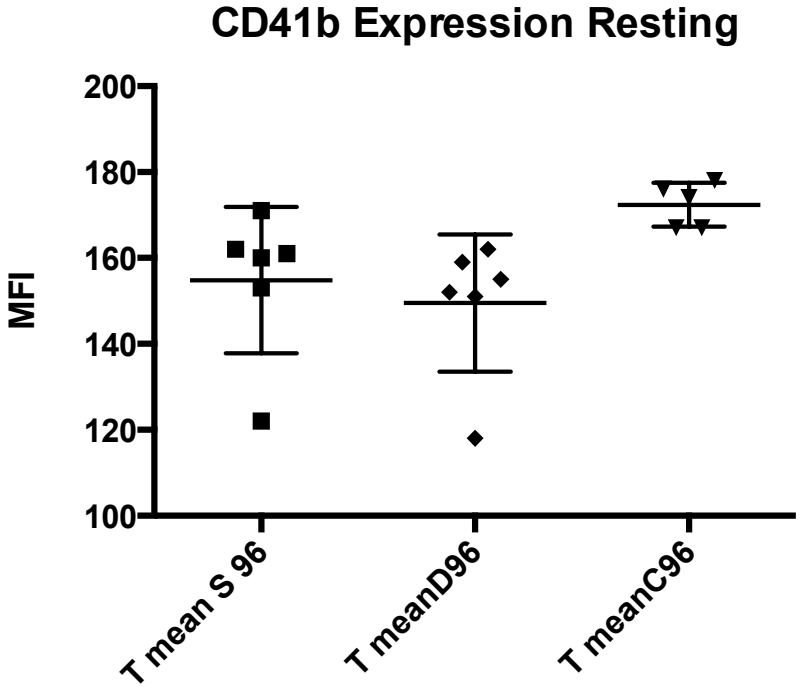


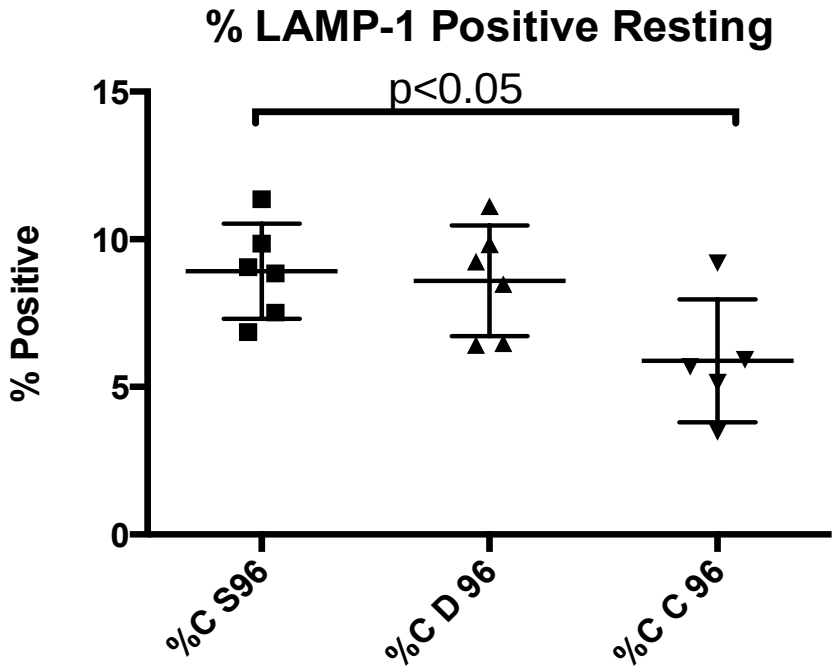


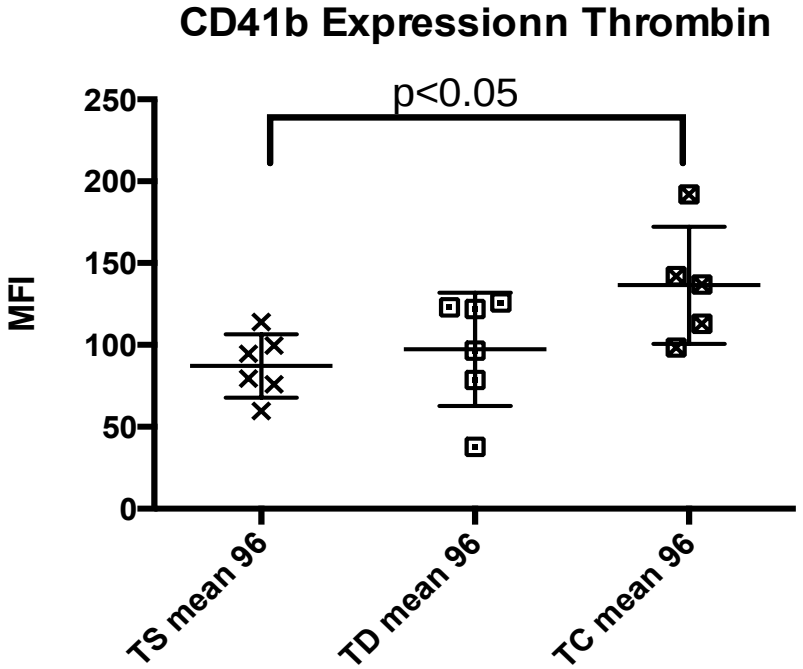
Time-Delay Study (Urban vs Rural)

Flow Cytometric Platelet Evaluation

96 hours post-exposure







Outline

- Study Motivation and Objectives (Dr. Pinkerton)
- PM Sample Collection, Extraction and Chemical Analysis (Dr. Bein)
  - Methodology
    - PM sampling; Filter extraction techniques; Chemical characterization
  - Comparative compositional analyses
    - Mass fractions; Mass closure; Retrospective mass reconciliation; Enrichment factors
- Animal Exposure Studies (Drs. Pinkerton and Tablin)
  - Animal exposure methods
  - Toxicological assay methods
  - Dose-response (PM extraction inter-comparison)
    - BALF, histologic evaluation, gene expression, immunohistochemistry, platelets
  - Time-delay (Urban versus Rural)
    - BALF, histologic evaluation, gene expression, multiplex cytokine assays, platelets
- Conclusions (Drs. Bein and Pinkerton)
  - PM extraction and chemical analysis
  - Dose-response
  - Time-delay

Conclusions
PM Extraction and Chemical Analyses

- First study to demonstrate:
  - Exhaustive chemical characterization of single extract
  - Importance of directly characterizing PM extract in toxicological studies rather than parallel measurements
  - Relevance of directly measuring extracted PM mass rather than pre-/post-extraction filter weight difference
  - Substantial compositional biases between different filter extraction techniques
  - Importance of standardizing filter extraction objectives to minimize study bias in toxicological studies

Bein, KJ, and AS Wexler. Compositional variance in extracted particulate matter using different filter extraction techniques. *Atmospheric Environment*, 107: 23-34, 2015.

Conclusions
Dose-Response Study (MSE vs SDE)

- Filter extraction method critically influences biological responses observed following administration of extracted PM to the respiratory tract
- Pathology and inflammation associated changes more potent for spin-down extraction
- PAH related responses more potent for multi-solvent extraction

Van Winkle et al. Biological dose response to PM2.5: Effect of particle extraction method on platelet and lung responses. *Toxicological Sciences*, 143(2): 349-359, 2015.

Conclusions
Filter Extraction Paradox

- MSE best conserves original chemical composition of sampled PM
- SDE generally elicits largest and most robust toxicological response
  - Retrospectively determined to be at roughly half the intended dose
- What is the reason behind these observations?
  - Toxicological matrix effects
    - SDE removed toxicologically inert components, amplifying response to active ones
    - MSE maximized extraction of all components; inert ones dilute response to active ones
  - Altering physical composition of PM alters bioavailability
    - Particle size distribution: agglomeration, component dissolution…
    - Internal distribution of chemical components
    - Phase partitioning of chemical components: particulate, immiscible, dissolved
  - Presence and concentration of FGM greatly affects toxicological response
- Which method is more appropriate?
  - Depends on study objective
    - Conserve original physical and chemical composition of sampled PM
    - Produce largest and most robust toxicological response

Conclusions
Time-Delay Study (Urban vs Rural)

- Significant shifts in temporal pattern of response based on particle type
- Urban PM has longer period of BALF inflammation and pathology associated with lung tissue inflammation
- Intriguing differences by lung region in terms of antioxidant and chemokine responses in the lung
- Novel increase in eotaxin in lung tissue following significant lag time after exposure

Recommendations for Future Work

- Reproducibility?
  - Different PM types, extraction methods, exposure models…
- Better understand effects of extraction method on PM toxicity
  - Toxicological matrix effects, bioavailability…
- Evaluate alternative filter media for PM toxicity testing
  - Minimize toxicological artifacts of filter media
- Quantify differential toxicity
  - As a function of filter media
  - As a function of PM extraction technique
  - As a function of PM component
  - As a function of PM source
  - As a function of toxicological endpoint
- Standardize filter media and extraction technique